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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIVI-GAG, POL. NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



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According to International Patent Classification (IPC) or to both national class	ssification and IPC	1	
B. FIELDS SEARCHED			
Minimum documentation searched (classification system followed by classific U.S.: 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 32.	cation symbols) (20.1, 456; 530/23.72;		
Documentation searched other than minimum documentation to the extent that	it such documents are include	d in the fields searched	
Electronic data base consulted during the international search (name of data be Please See Continuation Sheet	pase and, where practicable, s	search terms used)	
C. DOCUMENTS CONSIDERED TO BE RELEVANT			
Category * Citation of document, with indication, where appropriate, or	of the relevant passages	Relevant to claim No.	
X WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996),		1-3, 8-11, 18	
Y and claims 1 and 5.		4, 5, 13-17, 29, 30, 32, 34, 35, 37	
X US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000).	see columns 2, 7 and 8.	1-3, 8-11, 18	
Y		4, 5, 13-17, 29, 30, 32, 34, 35, 37	
X.P US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/20 and claim 1.	US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.		
X US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see e	examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18	
Y		4,5,13-17, 29, 30, 32, 34, 35, 37	
Y WANG et al. The use of an E1-deleted, replication -defective a expressing the rabies virus glycoprotein for early vaccination of Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-368	of mice against rabies virus.	1-3, 9-11, 13-18	
Further documents are listed in the continuation of Box C.	See patent family annex.		
	later document published after the inte date and not in conflict with the applic principle or theory underlying the inv	cation but cited to understand the	
of particular relevance "X" "E" earlier application or patent published on or after the international filing date	document of particular relevance; the considered novel or cannot be considered	claimed invention cannot be	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as "Y"	when the document is taken alone document of particular relevance; the considered to involve an inventive ste		
	combined with one or more other such being obvious to a person skilled in the		
"P" document published prior to the international filing date but later than the priority date claimed	document member of the same patent	family	
Date of the actual completion of the international search Date of many accompany	ailing of the international sea	rch report	
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Form PCT/ISA/210 (second sheet) (July 1998)		T	

International application No.

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INTERNATIONAL SEARCH REPORT

stešouh .	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type ! (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
	·	
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International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)	
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:	
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:	
Claim Nos.: 31 because they relate to parts of the international application that do not comply with the prescribed requirements such an extent that no meaningful international search can be carried out, specifically: This claim could not be searched because applicant did not provide a CRF.	
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).	
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)	
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet	
 As all required additional search fees were timely paid by the applicant, this international search report covers al searchable claims. 	
 As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invit payment of any additional fee. 	
As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:	
*	
No required additional search fees were timely paid by the applicant. Consequently, this international search reprise restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 35, 37	1
Remark on Protest The additional search fees were accompanied by the applicant's protest.	
No protest accompanied the payment of additional search fees.	

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immane response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>\Delta E1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

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		64
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
•		and AE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
		The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
15	55	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		and AE3, the vector contains the cis-acting packaging sequence of the waterpe
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
17	02, 03, 00	to HIV Pol protein with the recombinant adenoviral particle.
		The claim is directed to a method of generating a cellular mediated immune response
18	63, 64	The claim is directed to a method of generating a centual mediated minimal response
		to HIV Pol protein with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	AE1, the vector contains the cis-acting packaging sequence of the wild type
	13, 13	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
		The claims are directed to an adenoviral vector that is at least partially deleted of
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least part daily defected of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	ΔΕΙ, the vector contains the cis-acting packaging sequence of the wild type
	ļ	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	ł	inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	AE1, the vector contains the cis-acting packaging sequence of the wild type
	75, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
		The claims are directed to an adenoviral vector that is at least partially deleted of
22	67-70, 72,	The claims are directed to an adeliovital vector that is at least partially defected or
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
		The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
24	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		the vector contains the cis-acting packaging sequence of the wife type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		the antiparallel orientation of E1.
26		The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
26	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		the vector contains the cis-acting packaging sequence of the wild type adenovates
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
		and AE3, the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
		INDICATE IN The Advantage of a produced sension that is at least postially deleted of AE1
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	ŀ	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	1	inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
. /4	74	The clams is discount to the country of the country
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in E1.
	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
30	17	and AE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
	76-80	The claims are directed to a method of making and harvesting of a recombinant
31	76-80	adenoviral particle that contains a gene encoding an HIV Nef protein.
	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
32	81. 84, 65	response to HIV Nef with the recombinant adenoviral particle.
		The claims are directed to a method of generating a cellular mediated immune
33	82, 83	response to HIV Nef with the recombinant adenoviral particle in addition to
	1	administering a DNA plasmid vaccine.
		The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
34	86a	
		from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
••		from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
50		from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
31	00-4	from two individual vectors, one expressing gag-pol fusion and one expressing nef.
	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
38	300, 00	from two individual vectors, one expressing nef-gag fusion and one expressing pol.
	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
39	001, 00	from a single vectors as a fusion protein.
	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
40	80g, 80	from two individual vectors.
	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
41	800, 80, 69	individually from one vector.
		The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
42	86i, 88	from two individual vectors.
		The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
43	86j, 88, 89	
		from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
		individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
		individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as
		fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as
7,	0000, 00	fusion protein from one vector.
_		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as
48	860, 88	The claims are grawn to a militivatent vaccine wherein he and gag are expressed as

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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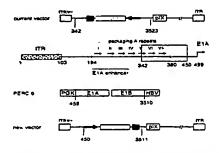
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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show en-A hanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HTV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.





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TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15 Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HTV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

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Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The tat gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

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The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

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Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HTV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8+ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

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European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, J. Biol. Chem. 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol*. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

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The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

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The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

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Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

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In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

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The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

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It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

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It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors. It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-

3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

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"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

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In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

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"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BgI*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

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Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

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Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Ins (A) and V1Ins-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

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Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3⁺ cells that were CD8⁺γIFN⁺ and CD4⁺γIFN⁺, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

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DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

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A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

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As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

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The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

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A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1 gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID 35 NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

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Along with the improved MRKAd5gag adenovirus vaccine vector described 15 herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). 20 Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent 25 or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with 30 one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the 35 MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

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The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine
series in a prime/boost vaccination schedule. This prime/boost schedule may include
any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine
series disclosed herein. In addition, a prime/boost regime may also involve other viral
and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine
vector regime includes but is not limited to plasmid DNA vaccines, especially DNA
plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

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Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

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Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

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Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

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Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6® cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

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The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of 1×10^{7} to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

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This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIVgag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the Bg/III recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1InsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

35 integrity.

is designated pVIInsCMV(no intron).

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

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EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901 ^a	10.8
PVIJns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPA ^{b,c}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

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EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA	Dose, ug ^b		Anti-p24 Titers (3 Wk PD1)°			SFC/10^6 Cells (4 Wk PD1) ^d	
Promoter/terminator		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)
Naīve	0	123	50	36	0 .	0	ó

in PBS

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Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).
- These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 µL per quad

cn=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

PCT/US01/28861 WO 02/22080

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each 5 reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 10 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

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EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

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EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml TerrificTM broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH₂0. A 2 μl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

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EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HTV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

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EXAMPLE 12

Stability Analyses

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To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed. under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

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Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Figag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

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EXAMPLE 13

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Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

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Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

^{*} This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

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Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10° ca0s/r	ni), Viability (%)	Harvest Time	Cell Passage	Titer	Tites	QPA	Patio	Amplification	AEX
	Infection	Harvest	hpl	Number	10 st vp/ml culture	10° vp/ce3	10° TCID _{so} /mi	AEX:QPA	Ratto	Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.78, 59%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 94%	0.88, 64%	47.5	54	9.0	8.7	1.10	82	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4,4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1.05, 66%	47.5	58	3.0	2.8	1.16	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2,86 2,60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	5.11	52	210	3.18 3.18
P14	1.94, 92%	0.88, 67%	46	63	8.6	4.4			160	3,2B 3,27
P15	0.97, 95%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° cells/n	nl), Vlability (%)	Harvest Time	Cell Passage	Titer	Ther	QPA	Ratio	Amplification	AEX
	Intection	Harvest	hpl	Number	10 ¹⁰ vp/ml culture	10° vp/cett	10° TCID _{sc} /ml	AEX:QPA	Ratio	Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MQI = 125)	
P5	0.92, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1.55, 88%	1.26, 76%	49.5	50	1,2	0.8	0.56	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	58	2.1	2.1	0.47	45	75	3.12 2.84
P9	1.20, 89%	1,26, 61%	47.5	58	0.8	0.7	0.29	28	25	2,70 2,60
P10	0.99, 82%	1.55, 86%	47	60	2.3	2.3	0.43	53	80	2,70 2,70
PII	1.07, 98%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.96, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 95%	1.03, 98%	48.5	47	9.4	9.7			350	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

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MRKAd5gag(E3-)

		ni), Viability (%) Harvest	Harvest Time	Cell Passage Number	Titer 10 ¹⁹ vp/mi culture	Titer 10 ⁴ vp/cell	QPA 10° TCID _{co} /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	Infaction 1.82, 77%	1.12, 62%	h.p.l. 47.5	46	2.0	1.2	0.92	20	100	internal Contro
		1							(MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 95%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.86 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

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Viral Vectors ^a	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^c	0.42

^a A_{260nm} absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

²⁵ d Research Ad5FLgag lot# 6399

e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group	Vaccine	Dose	GMT	SE upper	SE lower
ID_		(vp)			
1 2	^a MRKAd5gag	10^7	25600	5877	4780
	"	10^9	409600	94028	76473
3 4	hCMV FL-gag bGHpA [E3-] →	10^7 10^9	7352 235253	2077 59767	1620 47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6		10^9	310419	99181	75165
7 8	^b mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
	"	10^9	941014	239068	190636
9	^c hCMV FL-gag bGHpA [E3-] ←	10^7 10^9	3676 117627	934 17491	745 15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12		10^7	14703	5274	3882
13		10^8	58813	14942	11915
14		10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16		10^7	4222	3405	1138
17		10^8	19401	3939	3274
18		10^9	89144	25187	19639
19	Naïve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

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^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) ws used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MR KAd5gog ^a , 10^11 vp						- 3		
97N010	<10	118	5528_	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845_	3719	ND	24031
MRKAd5gag, 10^9 vp								
97N120	<10_	51	204	318	366	482	ND_	6550
97N144	<10_	18	118	274	706	888	ND_	7136
98X008	<10	15	444	386	996	1072_	ND	12851
Ad5gag ^b , Clinical Lot, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604_	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	<10	<10	143_	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558_	ND	11861
MRKAd5gag (hCMV, bGHpA, E3+)		<u> </u>	<u>l:</u>	}				
barlgind Adagag vector (hCMV/intro	n A bGHp	A. E3-), lott	FN0001_	<u> </u>				
ND, not determined	<u> </u>	L		<u> </u>			<u> </u>	L

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp#	Vaccination	Monkey ID		WK		Wk	[=1	l WK	1=1	6 WK		5 Wk		WK_
	T=0,4,25 wks		Media	Gog H ^b	Media	Gog H	Media	Gog H	Media	Gag H	Media	Gog H	Media	Gogl
			١.		۰	395		1058	0	1174	3	775	4	1074
1	MRKActigog	97N010	6	89	١٠	333	0 3	993	U	11/4	ő	76	Ö	594
	1041 vp	97N010(CD4-)	1 4	38 396	1 1	609	ا ة	534	4	395	ıï	261	ŏ	408
		97N116			١,	009	0	593	•	373	٠.	184	ŏ	666
		97N116(CD4-)	111	676 579		1304		2193	1	2118	3	1588	ŏ	2113
		98X007	10		0	1304	3		' '	2118	0	1656	ŏ	127
		98X007(CD4-)	20	965			0	2675			١ ٠	1000	0	12/
2	MRKAd6gog	97N120	5	275	1	249	4	141	4	119	9	206	4	219
	10/9 VD	97N120(CD4-)	11	170			0	85			0	75	1 1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			9	NO	0	625
		98X008	4	368	1 1	1090	3	891	4	673	3	473	5	73
		98X008(CD4-)	14	696			0	1175			٥	391	4	848
3	Actigog clinical lat	97X001	6	261	1	485	0	817	0	1220b	1	894	0	185
•	10/11 vo	97X001(CD4-)	10	283			3	996			0	1010	0	112
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	178
		97N146(CD4-)	اها	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	174
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ac5gcg dinlad lat	97N020	3	30	1	101	0	66	0	36	0	26	0	41
7	10/9 VD	97N020(CD4-)	10	29			0	15			0	1:	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1	12
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Nave	96R041	6	8	1	1	0	0	0	0	0	0	1	0
-		053F	14	18	5	16.	20	14	19	15	10	15	24	9

Basedion either 4x10/6 or 2x10/6 cells per well (depending on spot density)

ND, not determined

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⁹mock or no peptide control

^bPool of 20-aca peptides overlanding by 10 ac and encompassing the gag sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

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A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
•	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCT	GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 10 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp 15 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln 🕔 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 25 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 35 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp . 25 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

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DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at 10 least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 15 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any 20 combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type 25 amino acid with an alternative amino acid residue.

Table 1

	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187.	Ala	RT
30	Asp	188	Ala	RT
	Asp -	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

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AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG 10 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTCC ACAGACAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

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In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala 25 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys 30 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

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To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5'end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGT CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT

35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA 20 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG 30 GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

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Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 25 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr. Glu. Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu 35 Ala, Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 10 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly 15 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

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The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG .25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA -GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT 15 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

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Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEO ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

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1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA

GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG

CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA

ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG

GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC

TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC

AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT

ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC

CCGTGGAGCC CGAGAAGGTG GAGGAGCCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC

CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT

CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT

AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

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HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

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Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG 25 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC CCAGCACGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC (SEQ ID N0:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val 10 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp 15 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His 20 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. 25

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

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GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGGCGT GAGGAGGACC GAGCCCGCCG CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTC CAGAAGAGGC ACGACACTCT GGACCTTCCCCGG CTCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGGC GAGGAAGGT GAGGAGGCC ACGAGGGCGA GAACAACTGC GCCGCCCACC CCATGTCCCA GCACGGCATC GAGGAGCCCA ACGAGGGGG GAACAACTGC GCCGCCCACC CCAAGCTGGC CTTCCACCAC GAGGACCCC GAGAAGGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C CSEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

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15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

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sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG 5 GATGAGGAGG GCCGACCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA CTTCCTGAAG GAGAAGGGCG GCCTGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC CCAGCACGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC (SEQ ID NO:15).

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The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu 25 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a 35 deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

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EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid of the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

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Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

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MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Sca1. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone

plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

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Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the $Bgl \coprod$ sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with $Bgl \coprod$ and the gag reporter gene ($Bgl \coprod$ fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl \ \Pi$ site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by $Bgl \ \Pi$ digestion.

EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

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Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac1* and *BstZ110I* digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla I* digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Ins-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 μg/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFy-secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5×10^6 /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μ L/well of either 5 μ g/mL purified rat anti-mouse IFN- γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN- γ IgG2₂ (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁴7 vp. The humoral responses are highly dosedependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				Ал	ti-RT IgG Tite	az,	S	FC/10^6 cell	ls°
Gronb	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2 1 ·	1638400 ^b 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2 1	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2807(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2	1638400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

*GMT, geometric mean titler of the cohort of 5 mice; SE, standard error of the gemetric mean

^bNear or at the upper limit of the serial dilution; hence, could be greater than this value

No. of Spot-forming Cells per million spleonoytes; mean values of triplicates are reported along with standard errors in parenthesis.

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11 Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				Aı	nti-nef IgG Tite	ers"	S	FC/10^6 cell	s ^b
Group	Vaccine	Dose -	No. of Doses	GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10^9 vp	2	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAdSmCMVFLnef (E3+)	10^7 vp	2	132 115	42 45	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132 100	42 0	. 32	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52 ·	21(2)	18(6)	26(3)

*GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

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Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

No. of spot-forming cells per million spiechoytes; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

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Vaccine (T=0.4 wks)	Monk #		Prebleec	l .		_ T=4			T=7			T=16	
		Mock	PolL	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	PolL	Pol R
MRKAd5hCMV-lApal(E3+) 10^11 vp	99C100 99C215	1	0 2	0 2	1 10	38 98	31 249	0	52 109	148 305	0 22	49 88	715 250
	99D201	5	5	4	6	149	95	0	40	35	0	35	18
MRKAd5hCMV-lApd(E3+) 10/9 vp	99D212 99D180 99C201	0	2 4 5	0 2 21	4 0 6	331 19 62	114 192 82	0 4 0	58 36 18	14 156 32	0 5 1	6 38 14	6 106 65
MRKAdShCMV-IApol(E3-) 10^11 vp	99D239 99C186 99C084	5 4 1	2 12 8	2 6 9	20 5 8	82 120 84	172 421 484	1 2 0	66 271 14	114 489 238	9 16 1	21 875 24	40 530 264
MRKAd5hCMV-IApal(E3-) 10/9 vp	007C 0016 0011	10 2 6	10 0 6	8 1 12	12 5 10	724 474 98	745 468 110	4 0 5	322 232 60	376 212 80	4 0 8	188 101 25	176 121 34
Nove	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined Reported are SFC per million PEMCs; mean of dublicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN MMU	fml.			
Vaccine/Monkey Tag	T=4	T =7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRK Ad5hCMV-IApol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
990084	235	2637	2858	1626_
MRKAd5hCMV-IApol(E3-), 10^9 vp		<u>.</u>		
CC7C	32	175	306	235
മാഭ	20	140	273	419
©11	15	112	149	237
	!			

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

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Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	P	re	T:	= 4	T:	=7	T≘	16
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10/9 vp	CD15	5	4	30	998	2	586	0	434
·	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
	99C193	1 1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10^9 vp	99D250	8	_9	4	108	0	54	0	5
•	99C120	1	6	20	299	0	92	.0	79
Naîve	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-20 b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular 25 immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15

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Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
		(from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99		5	1055	1080	2210	2140

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer	AEX Titer	Amplification
	(10 ¹⁰ vp/ml culture)	(10 ⁴ vp/cell)	Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

			O' cells/ml), ity (%) Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 10° vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%	_	62	0.8	0.7	25	1.6
	1 2		0.99, 62% 1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1 2		1.22, 70% 1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

			0 ⁶ cells/ml), ity (%)	Cell Passage	AEX Titer (Cell Associated)	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10 ⁴ vp/cell	Ratio	10 to vp/ml culture
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
			0.96, 70%					
	2		1.18,73%	1				
bCMV-FL-pol [B3+]	`Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER C6[©] cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

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Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	-	Xv (10 ^s cells/m	d), Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	104 vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		. 60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					-
	2		1.34, 74%	ŀ	!			
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	· 75	4.6
	1		1.49, 84%					_
ł	2		1.18,77%					

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EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C	
DO	30%	·
PH	7.30	
Agitation	150 rpm	
Sparging	None	•

Table 21: Virus source used for experiments.

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Run	Batch ID	Cloned/Uncloned	MOI
		MRKAd5nef	(vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

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Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 ¹³ vp/L)					
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate		
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76		
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46		
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88		
	B20010202-2	Cloned	0.50	6.00	6.50	8.47		

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 ¹¹ IU/L)					
		MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate	
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28	
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86	
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89	
1	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47	

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

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Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

336 755 385

45 828 828 183 828 15

859 1916 836 1549 1229 25 88 85 872 2278 888 872 35 35 65 38838 85886 \$ 5 8 2 8 8 2 8 2 8 989 H 224 58 68 46 270 164 530 530 530 530 82228 284 288 118 85 t 28 825528 8 t = 8 t | Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag
Number of SFC/million PBMCs	Boost	Monke	Table wis	Medium
Table 34,8 wks	Medium	Table wks	Medium	
DNA/5 mgs	MRKAd5gag(E3+)	CBSH	NA	
DNA/5 mgs	10/7 vp	CCBX	0	
(D101)	AW3G	5	040∑0 CC1C CC1K AW3P CB6F AKBB AW20 CA4R CB5W CB5W CB7D MHKAd5gag(E3+) 10v7 vp MFKAd5gap(E3+) 10*7 vp DNA/5 mgs+ CRL1005/7.5 mgs + 0.6 mM BAK DNAV6mgs + CRL1005/45mgs 4 NA, not available	

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

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The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

HIV-1 gag, pol, gagpol, nef in rhesus macaques

Grp#	Vaccine	Monk #			T=6 wks		
	T=0, 4 wks		Mock	Gag H	Pol - 1	Pol - 2	Net
1	MRKAd5 gag	CB9V	0	15	- `	-	-
- 1	10^10 vp	CD19	0 .	374	-	-	-
		109H	1	843	•	•	-
2	MRKAd5 gag	99D130	1	948	-	-	-
1	10^8 vp	W277	16	324	-	-	-
		143H	4	595	-	- '	•
3	MRKAd5 pol	CC1X	4	-	46	256	-
- 1	10^10 vp	AW3W	3	- ,	463	550	-
		AV43	6	- /	95	1333	•
4	MRKAd5 pol	AW38	1	-	19	30	-
- 1	10^8 vp	CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef	076Q	9	-	-		120
	10^10 vp	091Q	4	-	-	-	85
		083Q	0	-	-	-	17
6	MRKAd5 nef	00C029	1	•	-	-	114
- 1	10^8 vp	98D022	6	-	-	- 1	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	24
	10^8 vp each	81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	72
Ì	10^10 vp each	22H	4	385	119	1194	191
		61H	4	343	11	765	853
10.	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
ļ	10^8 vp each	48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

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A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- 2. A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- A vector in accordance with claim 1 further comprising nucleotides
 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
 - A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;

- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the geneexpression cassette is in an E1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
 - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
 line which expresses adenovirus E1 protein at complementing levels.
 - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises a physiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

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- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
- 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
 - 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

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- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
 - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
 - 39. An HTV vaccine composition comprising purified adenovirus particles of claim 38.
 - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 20 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6[®] cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

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- b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.

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- 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
- 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
- 61. A method according to claim 60 wherein the cell is a PER.C6[®] cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

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- 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
- 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
- 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
- 75. A cell comprising the adenoviral vector of claim 68.

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- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6[®] cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

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- 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - a) gag, pol, and nef, expressed independently from three individual vectors;

 b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

- c) gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

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n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
 - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

 10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

 operatively linked to a single promoter; and the encoding nucleic acid sequences

 operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

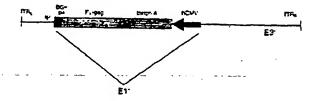


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggecetggagaagattgaggaggagcagaacaagtecaagaagaaggeceageaggetgetgetgee acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag gccatctcccccggaccctgaatgcctgggtgaaggtggaggagaaggccttctcccctgaggtgatccc catglicitgcctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagtggggggccatc aggetgecatgeagatgetgaaggagaceateaatgaggaggetgetgagtgggacaggetgeateetgtge acgetggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat ccigggccigaacaagatig:gaggatgtactcccccacciccatcciggacatcaggcagggccccaaggag cccticagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagagggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct agctglacccctggcctccctgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

Old Transgene: New Transgenes: CAG BGH CAG BGH CAG BGH CAG BGH CAG BGH CAG BGH CAG BGH

Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

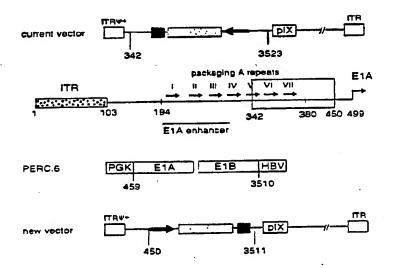


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

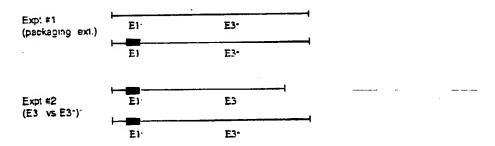


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

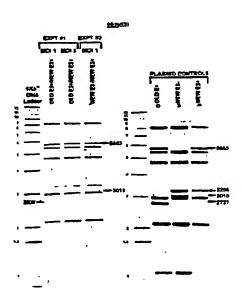


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

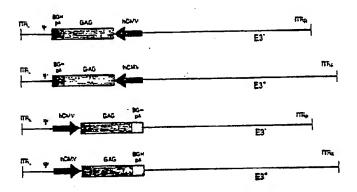


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

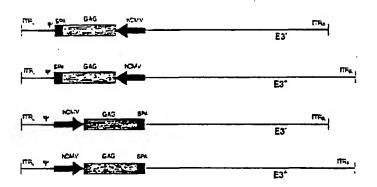


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

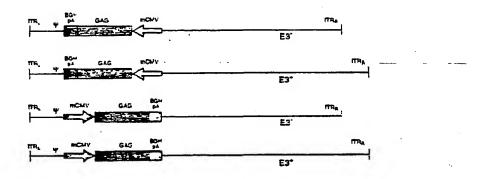


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the *MRK* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

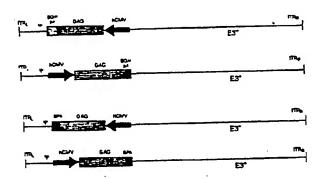


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

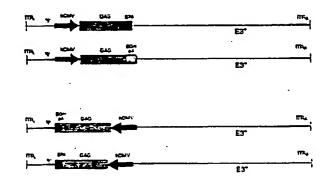


Figure 8B: Effect of polyadenylation signal

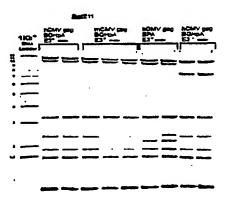


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.

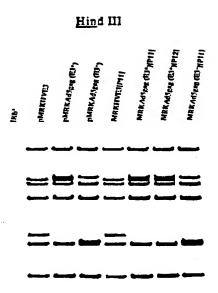


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

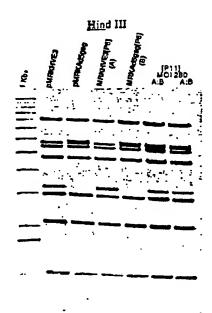


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).



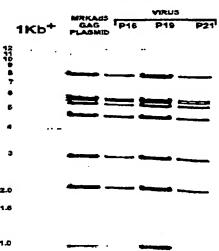
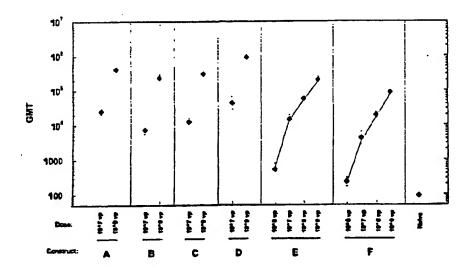


Figure 12: Viral DNA analysis by *HindIII* digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *HindIII*), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb's mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3* hCMV-FLgag-bGHpA; (C) MRKAd5 E3* bCMV-FLgag-SPA; (D) MRKAd5 E3* mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



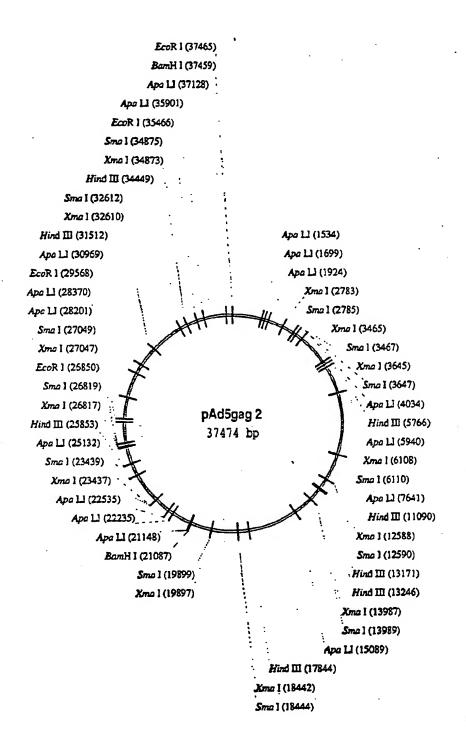


Figure 14

ACCAGAN' AA TOCTION ACCACAGACC GCTKIGAGAAGG CACCTGAGGT TINCTOCATA TCATTOTAGA" CCATICCGCAC CACACCCCC CTGTGGCCC COACCACTACA **CCTATAITA**I CATTAGTTCA GTENTCARGE AATCACCTAT ATAGTATAC NGTACATOTA GGTAN CGT TATCATATY ACAGGTRAGG TCTCCACCC CTCCATAGAA AGGGCTTCTG TCCCGAAGAC ATTICAGGAGG ACCTCCAGGG TAATGCCCCA GCCCAACGAC CCCCCCCAT TOACGTCAAT ACATCAAGTG TOTAGTTCAC GGATGAACCG GACTCACGO GATTFCCAAG CTANAGGTTC CCCCATTGAC GCAAATGGGC CGTTTACCCG CACAAACTO GAGGTATCTT CCTCCAGGGA GCACGETCCCT AGGCTCTGAG TCCGAGACTC TAACTCCTCC CCAMATGCAC ATCCAACATA ACTOCAGTITA ATGGGACTTT CCTACTTGGC ححوصوصحوه TACCHICHAT ATACTAATCA ATTACGGGGT COCCUCOCCCC TOACOFFFFF ACTGCANANA **GCCATTITICO** CGGTAAAAGC CULTITACGIO CCTGGAGAAG ATTOTOCAGA GOOGLANCIG CHGTTTTTCAC GTACCCACGA ATTIGRETICOG TAACACACCC CCCTCCAAAC COCACCTTTG **GCACCITCTTC** TAACACGTCT CATGGGTGCT CCTCAMCTO ATCCATTGCA TAGGTAACGT TATCATTAGE THUTCHCACGE AAACACTKICA GTGGCNNNG CACCGITITIC GTAAGATTTG CATTCTAMAC GGACTTTGAC GROCCOCOTA ACTICOCAGE TGAACCGTCA TACCCTIGNA CTCMCTGCCC CEGITENGATE GEETINGAGE GECATECACG CCAGATATAT TCGTCTCGAG CAAATCACTT GACAGTCTAG CGGACCTCTG CGGTAGGTGC GANCTACCCC ACATGACCTT ATGGGGGGGGG ATAGGGGGTTT AACAACTCCG CATTGOANCG COGATTCCCC GINCLAAGAG TRAGATCTAC CACKETTOTO ACTOTAGATO GETTAGA AGAAGTACAA GCTAAAGCAC CCATTICGTG GCAGGCAGAT CCTGGGCCAG CTCCAGCCCT GAGCTCGGGA CCANGGARGGC GGTTCCTCCG CTTCATCOGG CTATTTACKS TANACTISCCC TCTACTGGAA ATAMARCTA ACTIVYSITIN TACTATTACT CCCCACCTC TICATTATICA CTAGITATIA TATCCCCANA THGTTGAGGC CCAMATCCG CCTACAACAT CATTFAAACC CCCATTFGGCT Tecesses COCCCCCCCC 2020022002 GATCAATAAT CGGGTTGCTG ATTITICACING ACCUSACCIONT TOXINGULA CHATTANCOA AGRICCCARGG TACCCCCACC CATAMATICC TYTHEATHER CHARCCCCCTC GITTANIACACA CACTFICCTIGT AGGREGA TCCACAGGGT TATTATTATA TATGCCCAGT ATACCCCTCA AAAATGTCGT TTTTINCAGCA ATCATAATKIA TCACACCGCC TIGTGTACAT CITABATITITICS TRIPOTERACTIC ATACACATAN ATATETICITY TATAMCAGA **ATMTANTAT** ACTAATAACT כדנינירדיאכב GACCGACTOS AACACATGTA GTAACCTTGC GCCTAAGAGG CHETICACCA GAAGATTGAT TGGCACAGG AAPTYCAGGC TICCGGGTCG TCCGACGACG ACCGTCTCCG TTV:ACX:TCCG CGACTCCCGA CCACCGTTCT CTICTARCTA TCATGTCCAA CATTACCGCC ATGTTGACAT GGGACTITICC GTTTTAGTTG CCCTGAAAGG CGTCCGTCTA ACTIVITIVE ASCURE COTTTACCC CGATGITGEN TATEMERSON CTTKXCCCTTT CANCCACAM TACAACTGTA ANTEGECECIC TTACCGGGCG CATTGACCITY: AATGGGTGGA TTACCCACCT CCCCTCCCAT GCCGACCCTA CAGTACATCA GTCATGTAGT AGACTCCCCA GTGTCACCOA TOGGACATGA CACACGTCGT ACTIACGGTA GTTTAGICAM TCTCAGGGGT ACTTATTAMA ACACAATGAG THE CORRECTION TCAATGCCAT **OTANCTIGUAG** CTAMATOCCC CATTITACCOS CCCCTTTTCC COCCANANCE CAMATCANC GCTRAAGGGCT TYPATOTANCA ACTACAACGT ANACARGENA GOCCCAGTIT GTAATGGGGG TATTTICKGAT GOGMACACTA TCCCTGAAAG CAGTTACTGC GGTACCACTA CAAAACCGTG ACCAGAGCTC CCCTTGCCAC AGAAGATCAG TCTTICTAGEC GCTOGAGACC CGACCTCTGG ACCURATACT ACCITICATO GCGTTACATA ACCORNITION GTCAATCACG CCATCCTCAT GTTTTGGCAC COCMATGTAT TAATATACET ATTATATATA GGCGGAAACTG CCCCCTTCAC ATTTITUTE TAMAKKONO TGANTANTIT AGTACAGGTT MARGCCCAGC AATATAACCG ATCOCOTATA TACCTCAAGG TAACOCCAAT CAAGGOTATC ATTIGCGGTTA CCCTATTGAC GOGATAACTO ATCGCTATTA TACCGATAAT ATCCCACTT TACCCTCAAA GETCTATATA CHCCGCGGCC GAGGCGCCCCG GACAAGTGGG CTGTTCACCC Acceroacer TOCGACCOGA CACAGTGGCT ATCGAGTTCC AGAGTCCACA TTATATTGGC COMMUNICACA AACTGAAATC TTCACTITAG Tereaggigt TTCTTAATTA ACATCATCAA TYTACTACT GTAGTAGTGT CATCATCACA CCTTCACTGT GITCCCATAG GTTCATGCGG CCATAATICAG MOCCACCCT GOGACATGTT GTCCAAGAAG CAGGITTC1TC COTATTAGTC ATTOACCICA TAACTGCAGT TACCOCTOCOA CCGATCCAGC COCTAGGTCG TYCHOADCTO ACCACTCGAC PTTGCTGTGA MACGACACT CCCTGTACAA AACAATTAAT CACCAGGTOAC CCCCCACTG CHUTACACA GANTAAGAGG ATATGTACAT *PATACATGTA* **INCCCCATAT** CAAGTACGCC CCACATGROT CHTATTCTCC CAGGIGITIT GTCCACAAAA 1101 1301 1501 1601 1201 1401 701 801 901 1001 401 501 601 201 301 101

Figure ISA

1701	CACCAGGCCA	Terececes	GACCCTGAAT	GCCTCTCTCA	ACCEPTATION	נימאמאאממניכ	Therecette	ACCTGATCCC	CARBITICICT	GCCCTGTCTG
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1001	AGGTGCCAC TCCCACGGTG	CCCCCAGGAC	CTGAACACCA GACTTGHAST	TCC:ACTTCAACAC	ACM TO COOK	CANTACKETE GIPACTECENE	CCATCCACAT	GCTGAAGGAG	ACCATCAATG TOOTAGTTAC	AGGANGCTON TOCHTOGAE :
1901	TGAGTGGGAC	AGGCTGCATC	CHCFFFCALCC	TOGGCCCATT	נופלאלאטינינונו נופלאלאטינינונונונונונונונונונונונונונונונונונו	ACATICAGOGA	הריכראמנים ברימנים בר	TCTGACATTG	CTOCCACCAC	CTCCACCCT.
2001	CAGGAGCAGA	-		CCCCCCATCC	CHYTERIGORIA	ANTCTACAAG	ACTIONS TO	AGGACCCGGA	GAACAAGATT	GTCACCTACA
2101					CETCOGGAAG	ARGGACTATG	TGGACAGGTT	CTACAAGACC	CTGAGGGCTG	AGCANOCCT.
2201	CCAGGAGGTG		_		CAGAATGCCA	ACCCTGACTG TOGGACTGAC	CANGACCATC	CTGAAGGCCC	TGGGCCCTGC ACCCGGGACG	TOCCALICETED ACGETEGGA
2301	CICCICINCT	TOACAGCCTO	CCACCICCAC	GRAGOCICTES	CACTACAAGC	CAGGGTGCTG	GCTGAGGCCA	TOTCCCAGGT	GACCAACTCC	GCCACCATC, CGCTGGTAC!
2401	TGATOCAGAG	GOGCAACTTC CCCGTTGAAG	AGGAACCAGA TCCTTGGTCT	GGAAGACAGT	CTTCACGAAG	AACTGTGGCA	AGGTGGGCCA TCCACCCGGT	CATTGCCAAG	AACTGTAGGG TTGACATCCC	CCCCCAMBUM.
2501	GAAGGGCTGC	TOGANGTOTO	COTTCCTCC	CCACCAGATG	MONACTOCA	ATGAGAGGCA	CCGGTTGAAG	CTCCCCAAAA	TCTGGCCCTC AGACCGGGAG	CCACAAGOGI: GGTGTTCCCY:
2601	AGGCCTGGCA Treesgaccor	ACTTCCTCCA TGAAGGAGGT	GTCCAGGCCT CAGGTCCGGA	GAGCCCACAG	CCCCTCCCGA	CCTCAGGAAG	AGGTTTGGGG	ACCICITCITCIO	CACCCCAGC	CACMAGCAR!
					_					Dyfil
2701	AGCCCATTGA	CAAGGAGCTG	TACCCCTTG	CCTCCCTGAG	CAGGGACAAA	GREANTRACE	CCTCCTCCCA	CATITITATE	COCCCOORCAG	ANCTOCTOTA: TADACOACA
2801	CCTTCTAGTT				TGCCTTCCTT	CHEGGACETT	GGTGCCACTC	CCACTOTCCT	TICCTAATAA	ANTCACCAAN TTACTCCTTT
					Madamada				GACAATAGCA	Spirit
1067	ACCTAGEGY	-		GATAAGACCC	CCCACCCCAC				CTGTTATCGT	CCCTACGACC
		, •	¥ i	Asci	-					
3001	GRATOCOGTO CCTACOCCAC	GCCTCTATOG		CCGATCGGCG CGCCGTACTG GGCTAGCCGC GCGGCATGAC	AAATCTRETREE TTTACACACC	CACACCGAAT	AGGGTGGGAA TCCCACCCTT	AGAATATATA TCTTATATAT	AGCTCCCCCA TCCACCCCCA Sph1	CTTATGTAGT
3101	THETATETE	TTTTGCAGCA	ו פככפכפרכפ	CCATGAGGAG GGTACTGGTG	CAAÇTICATTT	מאדטהאאמכא כדאביבדניניד	TTEPGAGETE ANCACTEGAG	ATATTTCACA TATAAACTGT	ACCIOCIATOS TOCOCOTACO	CCCCATOCCC
3201	CECCACECA	T CAGAATGTGA A GTCTTACACT	ACCCGAGGTC	CATTGATTGT	מנונית בנינישבר מנונית בנינישנים	TGCCCGCAAA ACGGCGTTT	CTCTACTACC	TTGACCTACG AACTGGATGC	AGACCCTCTC TCTCGCACAG	TECATECOCE

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1301	AACCITCINGACIO	CARCCICCEC		GCCCCCAAGE CXXXXAATE (XIFXXXXXXX)						THYTICACCITC
1401	CITICCCOTIC	Arccocccs	CATCACAGE	TOACGGATCT					CAAAGACTICAGE	ACCITCITUMAN IN
	GANCIGICAAG	TAGGCDGGCG		CTACTCTTCA ACTUCCOACA	AAACCCTTTT				CAMMAGICA	TCCC CONT.
1501	TCTGCGCCAG			TRUTTURE				CCAGACTCTG	Triticaling	GATCAAGCAA
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,	CACAGAACGA	CACADAACGA CACAAATAAA	TCCCCAAAAC	CCTCCCCCCCA	TCCGGGCCCT	GCTCTCCAGA	٠	CCCAGGACAC	NTARARARGG	TCCTGCACCA
							Fish			
3701	AAAGGTGACT	CTOGATOTIC	AGATACATGG	GCATAAGGCC	GICTURO	GICTCITCION INSTABLE ACCACITICAG ACCITICATOC	ACCACTRICAG	ACCITICATEC		TCTTCTAGAT
	TTTCCACTGA			CGTATTCGGG	CAGATACCI C	ACCTICATEG	TOGTGACGTC	TCGAAGTACG		ACAACATETA
3801	GATCCAGTCG		CTONICCTO	GTGCCTAAAA	APCITUTERIA	GTAGCAAGCT		GGCAGGCCCT		CITTING AAN:
	CTAGGTCAGC			CACGGATTTT	TACAMAAAGT	CATEGITICGA	CTAACGGTCC	CCGTCCGGGA		CANATGITIE
1901	COCHTANGET	COCATOGGTO	CATACCITICAS	GATATGAGAT	GCATCTTAGA	CRSTATTITE				COCKERATIVA
	GCCAATTCGA		GTATGCACCC	CTATACTYTA	CGTWINNCCT	GACATANANA	TCCAACCGAT			GCCCCTANGT
4001	TOTTOTOCAD		ACAGTGTATC	CCGTCACACTT	GREAMATTE	TCATGTAGCT		TOCCITOGAAG		CONCRETERING
	ACAACACGTC				CCCTTTANAC	ACTACATEGA	ATCTTCCTTT			GCGGGAACAC
4101	ACCTCCAAGA		APPECITECAT	AATTATOGGA	ATTACACTOR	このいっというという	CTYCCCGANG			
	TOGAGGITCT	_	TANCCACCTA	TTACTACCGT	TACCGGGTG	تددوددودو	GACCCGCTTC	TATAAAGACC	CTAGTGATTO	CAGTATCAAC
4201	TOTTCCAGGA	TOAGATCGTC	ATMXXXCATT	TTTACABAGC	GCCACHCCGCAG	CHITCHOLONIA			COCCCVOOG	
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4101	CONTRACAGAT	-		CTTCAGATOG	CHASTATICATIC	TCTACCTGCG	GGGCGATTAAA	GAAAACGGTT	GAAAACOSTT TCCGGGGTAG	OCCACIATION
	OGACTOTOTA				CCCCTAGTAC	AGATGGACGC	CCCCCTACTT	CTTTTGCCAA	AGGCCCCATC	CCCTCTAGTC
										ISA Sees
4401	CTCCCAAGAA		TONOCAGETG	ACCAGOTICE TONGCAGETO CEACTINACES CARECTORITAS GEOEGENAAT CACACETAIT ACCAGETICA	CACCYGGTTG	GCCCGTANT	CACACCTATT	ACCOCCTOCA		
	GACCCITICITY		ACTCGTCGAC	GCTRAATGGC	מירכים	GCTVAATGGC CITCGGCCACC CGGCATTTA GTGTGGATAA	GTGTGGATAA	TOCCCGACGT	TGACCATCAA	Treference
4501	CAOCTOCCOT	CATCCCTGAG	CAGGGGGGCC	ACTICGITIM	GCATGTCCCT	ACTICOITAN GCARATCICT GACTORIANG TITICOCTOR CCANATCOOC	TTTCCCTGA	CCANATCCOC		
	GTCGACOOCA	GTAGOGACTC	: פוכנוננננננ	TCAAGCAATT	CGTACAGAGA	CTGAGGGTAC	AAAAGGACT SpH	GGTTTACCCC	GICTITCCGCG	AGCTAGCGGGT
4601		DESCRIPTION :	CAACCTAAACT	TETTICANCES		PTTGAGACCG TOCGCCCTAG	3 5	GACCOTFICA	CCAAGCAGTT	CCAGGCGGTC
100	CCCTATCOTC					ANTRICATOR				-
4701	CCACACCTCG							THICCCTOTA	COCCAGTAGT	CONTRICTOR
	GGTGTCGAGC									
4801	CCAGACGGG		TETTTCCACC	י מאראראראניד רבייראראניד	רכיונייייייייייייייייייייייייייייייייייי	יידאקידניזההם	AGTOCOLOT	CCCCACGCGA	מככבבמעכטב	CCCACCGGT"
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figure 150

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GPCCAGGGG CAGGTCGGGG GCGAGAAATA CGCTCTTTAT AAAGGAGGT TTTGGTCCA	ATCTCTCAN: AAGGAGGCTA TTCCTCGAT	ACTAACCANA GTCTGCGAGG CAGACGCTCC	TTCACCAGGY AAGTGGACCY GAGGCGTTYGGACCY GAGGCGTTYGGA	CCCCCAACCT AACGCACCK TTGCGTGGCJ	ACCTCTCCGT TOCIACIOGGT COTCCACGGTT OCAGGTTCCCA		TALGGGGGGG ATGCCCGCC OAGACTTACC CTCTGGATGT
ACCCCAGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	ACAGGGGAT ATGTCTGAN; GGCCAGCAC AAGGACGCTA CCGGTCGTC TTCCTCCGAT	TCAAGGAAGG AGTTCCTTCC CCGCATCGCT GGCGTAGCGA	CCTAAACTAT	CTGGGCATCT ATTCGCGCGC TAAGCGCGCG	CCCCCAGAC CCCCCAGAC CCCCCCAGAC CCCCCCAGAC		CCGAGOTTOC GCCTCCAACG TGGCGTCTGT ACCGCAGACA
GATTTOACCA GTAACTCOTA ACTCCCGCAT CTCTGGCCGT GAGACCGGCA	AGGCTGTCCG TCCGACAGGC CTCGCGTCCA GAGCGCAGGT	CTCTTCGGCA GAGAAGCCGT TCACTCTCTT AGTGAGAGAA	AAAACGAGGA TITTIGCTCCT	CCACCOTITIO ACCTACACOT TCAACOTICA	CANOCICAAC GITCCAGITG COTCTCGTCC GCAGAGCAGG		OAGOTCGGA CTCCAGCCCT ACGTTGAAGC TGCAACTTCG
GECCACTING CCCGCTCCATC TIGAGACTIT ACGICTICIMA GCCCACTICA	GCTCACGAAA CCACTGCTTT GAGACAAAGG CTCTGTTTCC	ACATGICGCC TOTACAGCGG GCGTTCGTCC CCCAAGCAGG	ACTCAAAGGT TTTTGCTCCT Hindil				ARGESTRANG ACCESTRANG TOCGACCETC
CETTACTICATE CENTACTICATE CENTA		CACACTTCTG GAGTGGGGGC CACACCCTCG	GCTAAGATTIS				AGTTCGTGGG TCAMGCACGC ATATGGTTGG TATACCAACC
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TYCTGOTGCT ACTACCAGGA GCGCAGCTTG CGCGTCGAC TCCGCGCGCG	GTPTCTTACC CAMGANTGG TGTTCCACGG	TREFCCACTA AACAGGIGAT CGGGIGITCC GCCCACAAGG	CTCCCTCTGA		CUCCCAAAC GCGCTCGTCG CCCGAGCAGC		
AGGCTORTCC TCCGACCAGG GGCCCTTTGGC CCGGGAACCG GGAGTAGGCA CCTCATCCGT	AAAAACTACG Xhol Xhol CCTCGAGGGG	GTAGCGGTCG CATCGCCAGC GCCACGTGAC CGGTGCACTG	GCCCACTCAT	GCCTTTGAGG CGGAAACTCC GCGATGGAGC	_	GOCAGCAGO CCGTCGTCG GOGAACCCA	
OCTGCGCTTG CCACGCGAC TCCGCGGCT AGGCGCCGCA CCGATCCCGG GGCTAAGGCC	TCCCCCATGC AGGGGGTACG AGGGGCTGT AGAGGCCTGT	AGTGGGAGGG TCACCCTCCC GTAGGTGTAG	OCCABCTOTT	CCGCGGTQAP GGCGCCACTA CAGCAACTTG	GTCGTFGAAC CATTCGGGAA GTAAGCCCTT GTAGGCGCTC	CATCCCCUAR AAAQACCCCG TTTCTOGGGC GGGTTGAGTG	ATOTAGGOTA TACATCCCAT CTGCTCTGCT GACGAGACGA
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Figure 150

6501	COCAGTCACOCA CO	CCANGGAGGC	GTACKINGTCG CATCKINGAGC	CACAGCTTGT	TGACCAGCTC ACTGGTCGAG	הההרימית הארוב כריה היי אנידה מ	Treassant	CCCCCCACTA C	CAGGTCCCAA	TCCTTGATGA
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6801	CACCACACCC AC	TGAGCGCAAA	GOTOTCCCTG	ACCATGACTT	TCIACKITACTY: ACTCCATCIAC	CATAMETEC	TCAGTGTCGT	CCCATCCGCC	CTGCTCCCAG	AGCAAAAAGT TCGTT!!TTCA
6901	CCOTCCCTT TT	PTTGGAACGC AAACCTTGCG	CCTANACCGT	CCCCCTTCCA	CRCATCCITY: ANGACTATCT CRCTARGAC TYCTCATAGA	AAGAGTATCT TTCTCATAGA	THY COCIOCO ANDOGOGOGO	ACCCEATAAAG TCCCTATTTC	TTGCGTGTGA	TOCKGANGGE ACCCCTTCCT
7001	TECEGOCACE TO AGGOCECTOO AG	TCGGAACGGT AGCCTTGCCA	TOTTANTTAC	CTGGGCGGCG	AGCACGATCT	CCTT AAAACCC GCA(FTTTCGG	GTTCATCITG CAACTACAAC	TCCCCCACAA	TETAAAGITC	CAAGAAGEGT GTTCTTURCT
7101	CCCTACGGGA AC	TCATCCAAGG ACTACCTTCC	CAATTTTTA	AGTTCCTCGT TCAAGGAGCA	AGGTGAGCTC	TTCACOCCAG ANGTECCECTE	CTCAGCCCCT	CCACACAAG	CCGGGTCAGA	GCAAGATY:AG COTTCTACT(:
7201	CCAACCTICG CI	CHCCATACTC	CTCCACAGGT	CACCCCCCAT	TAGCATTTGC ATCGTAAAAGG	ACCACCAGCG	GANAGETECET	ANACTOGGGA	CCTATOCCCA	TTTTTTCTGG AAAAAGACC
7301	CCACTACOTC AT	THCANOGTAN ATCTTCCATT	OCCOUNTETTO CGCCCAGAAC	TTCCCAGCGG AAGGGTCGCC	TCCCATCCAA AGGGTAGGTT	CCANOGCCG	TAGGACAGGG	COCCOTCAGE	CTAGAGGCTC GATCTCCGAG	ATCTCCGCCG TAGAGGCGGC
7401	AACTICATOA CO TTGAAGTACT GO PV	CCAGCATGAA GOTCOTACTT Pvd	OCCENCIACO CCCC	TRECTTECCAA	AGGCCCCCCAT TCCGGGGGTA	CCAASTATAS GCTTCATATC	GACAGATGTA	CCTARGTGAC	AAAGAGACGC 17TCTCTGCG	TCGGTGCGAA AGCCACGCT
7501	CHACGCICGO CHACGGAAG	CTAGCCCTTC	AACTGGATCT	CCCGCCACCA	ATTCCACACACACT TAACCTCCTC	TOCTATTCA ACCGATAACT	TCTCCTCAAA ACACCACTIT	OTAGAAGTEC CATETTEAGG	CTGCGACGGG GACGCTGCCC	CCGAACACTC
7601	CACGACCGAA AV	THOTAMAAC	OTOCCCAGTA CACGCGTCAT	CTCGCACACC	TCCACTAXCCT ACCTTXCCC()A	GTACATCCTG	CACCACCITIG	ACCTURACGAC COCOCACANO TOGACTOCTG (ACCOCATOFTIC	CGCGCACAAG	GAAGCAGAGF
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7801		CCCCCCCCAC						GCGCAGATGG	GAGCTOTCCA	TOGICTCOAN ACCAGACCTC
7901	CTCCCCCOCC OT GAGGGCGCCC CI	OPEAGGTCAG CAGTCCAGTC	OCCCTTCGAG	CHECACOTTY GACGTCCAAA	ACCITCOCATA		GACCAGUCAG GOCGCGAGCT CTGCCCAGUC CCGCGCCCGA	AGATECAGGE	GATACCTAAT	TTCCANGGGC.
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CCASACCCAC	CAACTAGAGG	מניביניניטאבניני כניביניניטאבניני	CCCCAGCGAG	הרככוברודים האסמאאת	TPGACKKFITES AACTCCCAGC	CCTYCTAGAA	CACCTCCCCC	GCGACACGGC	TrTCGCGGGG AGAGGGCCCC	CAACAATTRIT GITUSTIAACA	TCCCAAGGTA	TYPAGACTGCG ACT CTGCCGC	CACCACIANTE	CTCAAACCG	ATATOCCCOAC TATACCCCOAC
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AAGCGGTGAC	COACGCGCGC	GAAAGAGT	ATGAACTGCT	COTTORAGEC	GGCGAAGACG CCGCTTCTGC E	GATTCOTTCA	CCITCCTCCAG	CCGGA	00000		ATCGG	GCCCT CCCCT	00000	ACAG	
			2000	SANGO	MCOCC	ACGTO	TANCT	ATAAG	TCTCC AGAGG	20000	CATCUACCOS	TTCTO	TOAATGCGCA ACTTACGCGT	CTCCTTCCTC	TCATC
ATCCATCTA TACGTAGAT	ASSAGETAGE TCCTCGACC	GCTTGAACC CGAACTTGG	GATCTCGGCC	TOCCHOANGO	CCACOTOCCO	TCCCAACGT AGCGTTGCA	ACCONTANCT	CTTCCATAAG GAAGGTATTC	GATCATCTCC	GFTOCCOCC	CATCUACC	GHGTTTC	TOAAT	CTCCTTCCT	COCCOTON
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Figure15F

9701 ACAAAGCGGT GGTATGCGCC COTGTTGATG GTGTAAAGGC AGTTGACGAT AACAGAGTCT GGTGACGGG CTGCGAGAGC TCGGTGAAA	TOTTICGCA CCATACGGO GCACAACTAC CACATTICACS TCAACCCATA THICCHOSTC AATTGCCAA CCACTGAGCG GACACTCTGG AGCCACATAS	
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AGTTEGGCAT	TCAACCCRETA	
GTETAAGTER	CACATRIACE	
COTOTTGATG	GCACAACTAC	10
GGTATGCGCC	CCATACGCGG	Xhol
ACAAAGCGGT	TOTTTCGCCA	
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CCGCCATCTC	eccentaica
GGCCGCCGCA	TCCAGGTGAT
CANANAGIOC	TACCTOGACA
CA GRAGOCCTO GAGTOAATA COTAGENCET GEAAGHONG ACCARTANT GEAATOCCAC CAAAAATOC GGCGGGGGT GGGGFTAGAS CT CATTOGGGAG CECAGETTAT GCATCARAA CATTAGAGG TETTOGGGGG GTTETTCACG CGGCGGGA CGGCATCTC	COT ARGONDACES GOCTECTION GATCHARTAN CATCANTAN CATCANTAN TECTOTANA TACCTOCACA TECASOTION GEOGGENICA
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CTCAGTTTAT	BOBCTECHER
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CHICAGANTET	CCCCTCTAGA	CCASTITICTAGA	OCCANGGICT
COCCTCCAGG	CCCCAGGCCC	GTCACGGACG	CAGCGCCTYGC
ACCCITCCCCC	TCCCACCOC	CGCGCGGAAA	GCGCCCTTT
COCCCAOCGT	CCCGGTCGCA	GTGCTGCAGG	CACCACCTCC
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	10501		10601		10701		Luant		10001	10001		

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COCOCOCOCA CACGIGGCGG CCCCCGACCT CGTAACCTCA TACGATCAGA CGGGGANTCA GGAGATTAAC TITCAAAAAA CCTTTAACAA 9 GCCCCCGCGT GTGCACCGCC CACCGCTCAA CCATTGGCGT ATGGTTCTCT CCACTTCAT CCTCTAATTG AAAGTTTTT CGAAATTGT	OUT ACCEPTOTOUS CISCUIGIAGIA GOTSGOCPATA GRACTISATIC ATCHICTICARIA CITTICITANCIC GOGOTOGAGG AMARCICIAAA TAGGARACTIG SCA TOCGAMCACC GOGOCOTOCIT CONCCCATAT COTGACTANIG TAGARACCOT GRAACATICG COCGACOTOCA TITTIGGGITT ATCGITICGGG	CTCATOGIGG ACCIGITICET TATACITICAG CACAGEAGAGA ACAAFGAAGE, ATTCACEETA GEGACGATT TGTATCATCT COGGETECEG GCGACGAGG GAGTACCOG GCGACGAGTA TGTATCATCT COGGETECEG GCGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGACGATT TGTATCATCT COGGETECEG GCGACGACGACGACGACGACGACGACGACGACGACGACGA
GCACATTANC CCICTANTIG	GCGCTGGAGC	ACATAGTAGA TGTATCATCT
CCCTCANTCA	CTTTTTTANGE	COCCACCATT
TACGAGGAGA	ATCTCTCCCA	ATTEMOSTAT
CCATTGCCTTCA CCATTGCCCTT	CCTGACTACTAC	מטשטשארטע בובארופו
CCGCCGACCT	CCACCGATAT	CACAGEAGAG
CACATOGOOD	CICCICCACA	TATAGTGCAG
COCOCOCOCO	ACCCTTOTOO TOCGAACACC	ACCINITION TOGACAAGGA
11001 GGATTAGTCC	CCACCOTCCGF	CTCATGGGGCGC
11001	11101	11201

		2								
11301	TCGATTTCAT	AMCATECTS CAGAGGATAN		המהדיגראההא מרוציאתר דדה		עונירידנאמנידעי ו	ACAMOSTOGE	CCCCATCAAC	TATTCCATGC	TTAGCCTCCA
	AGCTAAACTA	TITICTAGGAC	CTCTCTCTATC	ACCACITECT	CULTARTANC	TUTEATHERAG	TUTTECACEG	GCGGTAGTTG	ATAAGGTACG	ANTERGACT
11401	CAAGITTTAC	GCCCGCAAGA	TATACCATAC	CCCTTACGTT	CUTATAGACA		ひいいひいいしい		CCATCCCCCT	CAAGGTTGCT .
	GTTCAAAATG	COGCGTTCT	ATATCCTATC	CCCAATCCAA	GGT:FAIT'N:T	TTTCATTT (CTARACTERICE	ANGATOTACO	CGTACCGCGA	כואניכעכני י
11501	ACCITIONGEG	ACCACCTOGG	CGTTTATCGC	AACGACACA	TEXTACTANGE	בנידהאיניהדה	אייכבטטריאמכ	GCGAGCTCAG	CONCCOCCIAG	CITTATICACA
	TGGAACTCGC	TOCTOGACCC	GCAANTANCO	TRUCTEGEGT	ACKTICITICS	פכעהבסמהעה	Traccere	COCTCGAGTC	geroscaere	GACTACOTATE
11601	GCCTGCAAAG	OCCCTOCCT	GGCATGGGCA	GCGGCCATAG	AGAGGGGAAG	TCCTACTTTG	ACGCGGGCGC	TRACCTERCEC	TGGGCCCCCAA	GCCGACTCG
•	COCACOTTIC	-	CCGINCCCCGT	CUCKYCIATE	TUTECOGCTC	AGGATGANAC	TOCGCCCCCC	ACTEGACOCO	ACCESSOOFF	COXXCHOCOC:
11701	CCTGGAGGCA	מכונפספככם	GACCTYCCCT	GREGGTRECA	בבבביניובנינים	CTCGCAACGT	CCCCCCCCTT	GACCANTATO	ACCAGGACGA	TCACTACGAG
	COACCICCOF		CTGGACCCGA	CCCCCACCGL	ეცეცეცენენ	GACCGTTGCA	GUCGCCGCAC	CTCCTTATAC	recreermen	ACTICATIGETE
					-				13:1	
11801	CCAGAGGACG	GCCAGTACTA	AGCGGTGATG	THETGATEA	CATCATGCAA		ACCORREGIONS		CTGCAGAGCC	AGCCOTCC()
	GENETICETICS		TCGCCACTAC	AAAGACTAGT	CTACTACGTT	CTRICITATION	TREGRECTRICA	כפכבכפכבפכ	GACGICTCOG	TCGGCAGGCr
11901	CCTTANCTCC	: ACGGACGACT	GCCCCAGGT	CATCGACCGC	ATCATGICGE	TICACTIVECACE	CAATCCTVAC	OCOTTOCOOC	AGCAGCCGCA	ממככתינכניו
	CONATTOROG		CCCCCCCTCCA	GTACCTGGCG	TACTACAGCG	ACTGACGCGC	GTTAGGM:TO	CCCANGOCCG	regreededt	ccoorrocc.
							Paril			
12001	CHATTER AA	THETHIGARINE	GGTOOTCCCO	GCGCGCGCAA	ACCCCACGCA	CGAGANGOTG	CTOGCGATKIG	TANACOCCCT	GGCCGAAAAC	ACCCCATOR.
10031	GAGAGGCGTT	_	CCACCAGGGC	COCOCOCOTT	TOGGGTGCGT	GCTCTTCCAC	GACCGCTARC	ATTITICCCCA	CCGGCTTTTG	TCCCGGrAG
12101	GROCCGACGA	_	GTCTACGACG	CHICTISCITICA	GCGCCTCGCT	CCTTACAACA	GUGGLAALGT	GCAGACCAAC	CTOSACCOGC	TOTOGOOGA
4	CCOCCTOCT	_	CAGATGCTGC	GCGACGAAGT	COCOCACCGA	GCANTISTICIT	CGCCGTTGCA	ceremogram	GACCTGGCCG	אככעככככנ
10001	CACCOCCO.	_	Accetdance	טטטטטטטטטטטטטט	CAGGGCAACC	TOCCCION	GGTTGCACTA	AACOCCTTCC	TOAGTACACA	OCCCCCCAN'
10221	ACACTACTACTACTACTACTACTACTACTACTACTACTAC		TCCCACTCGC	CCCCOTCGTC	GTCCCGTTGG	ACCCGAGGTA	CCANCGTGAT	TTGCGGAAGG	ACTICATIGITOF	COCCIONTE
10161		_	CTACACCAAC		CACTGCGGCT	AATGGTGACT	משעטעבעבבב	AAACTGAGGT	GTACCAGTCT	GCCCCAGACT
	CACOGCGCC	_	GATGTGGTTG	AMCACTCGC	CTCACCCCGA	TTACCACTGA	creterocos	TITICACTOCA	CATOGICAGA	CCCGGTCTGA
			Psil	_						
12401	ATTITITICCA	A GACCAGTAGA		CAACHICCTGC AGACCGTAAA	CCTGAGCCAG	CTTTCAMA	ACTIGCAMO	OCTOTOGGGG	GREGORGETE	CCACAGGGGA
	TAAAAAAGGT	r CTGGTCATCT	GINCCGGACG	TUTOCUTE	GGACTCGFTC	CHANGETT	TRANCOTOCO	CCIACACCCCC	CACGCCCGAG	CONCINCIO
12501	CCCCCCCACC	C GIGICTAGET	TOCTGACGCC	CAACTEGECEC	CTRITIONTIC	TUCTAATAGE	GUCCETCACG	GACAGTGGCA	GCOTGICCCG	GGACACATAC
	GOCCCCTGG	3 CACAGATCGA	ACGACTICICIO	CHITCAGCGCG	CACANCGACG	ACGATTATCG	CGCACAAGTGC	CTGTCACCGT	CCCACAGGGC	CCROIGIANS
12601		T TOCTOACACT	GTACCGCGNG	OCCATAGGGC	AGGCCCCATGT	CHALTAGEAT	NCTTTCCAGG	AGATTACAAG	TOTCAGCCGC	מכפבשמשענ
1			CATGGCGCTC	CGGTATCCAG	TCCGCGTACA	CCTGCTCGTA	THANGGICG	TCTAATGTTC	אכאסזכספכס	COCCUYCCCC
								*	Princip	
12701	ADGAGGACAC	C OCCARCCTG	GAGGCAACCC	TAAACTACCT		CONTROCAGA	AGATECECTE	GTTGCACAGT	OTTOCACAGT TTANACAGCG	ACCARRACTO
	recreetors	a ceedifedahe	Crccomman	ATTICATICA	רהאכיוראהידות	CCCCCCCCTCT	TETAGGGGAG			יייייייייייייייייייייייייייייייייייייי
12801	-	-					כאתכיהוממים	CTCCACATGA	ההתההההיא	GTACCTTGGC
	GTANAACGCG	O ATCCACGTCG	TCTCACTC	GGAATTGGAC	エルバスていいれいこ	CCCATTGCGG	מוכנצ:אנינוני	ניאניו ויוו ארו	סוור פריטיים	Olyce Control

Figure 15H

12001	CALL PROPERTY BARES	CONTRABACTO	THE PROPERTY OF	AACCCCCTAA	TEXTACTE	מייאדרינית לו	מנינטנינטניטעי	ACCCCCAGTA	TTTCACCAAT	GCCATCTTGA
10691							הטטטטטטעע.	THERESTEAT	MAGTGGTTA	CYXTAGAACT
	CCOMCAIRC						_			Brachman
13001	ACCCGCACTO	GCTACCGCCC	CCTONTITION	אנשננונונונונונו		C. V.GACKRETA J			CACAINGACG	The state of the s
	TOCCGTOAC	CGATGGCGGG	GGACCANAGA	בעבונוטכניככ	TAAGCTICCAC	CONCINCIONT TRICITACCITAR		SACCCTG	CTOTATCTGC	TOTOCOCACAA
							-	HingRID	•	
	S S LL	CITY BORDE	Tre-Transfer	נייאאריאיזיי	CAGENGGENG AGGCCCCT		CCCAAACCAA AGCTTCCOCA		GGCCNAGCAG	CTIGICCGAIL
13161	A MCCCCCCCA	CCOCMINGER	ACCONTCINA		כשכנישנינישנ		CUCTITICCTT TCGNAOGCGT		CCOOTTICGIC	GAACAGGCT,.
	10000000									
13201	CTACACTO	COCCCCOCO	GICAGATGCT	AGTACACCCAT		GATAGGGTCT	CTTACCAGCA	CTCCCACCAC	ccccccccccc	CTOCTORGO:
4	GATCCGCGAC		CAGTETACGA		AAGCTTCC:NA	CTATCCCAGA	GNATGGTCGT	GACCOTGGTG	0000000000	CACCACCC
			Psff	_}						
13301	ACCACACTA	CCTAAACAAC	TCGCTGCTGC	לבטכדאבינאנ: אשכנימכאמכים	CGAMMANG	כשמכבשבנימפ	CATTICCCAA	CAACGGGATA	GAGAGCCTAG	TOGACAN'AT
1	TCCTCCTCAT		AGCGACGACG	TCGGCGTCGC	GCTTTTTTG	GACTIGARICC	GTANAGRETT	GITGCCCTAT	CTCTCGGATC	ACC'TI TICTA
13401	CACTACATORS	AAGACGTACG	CGCAGGAGGA	CAGGGACGTG	CCAMPCTCC	CUCCUACCAC	COGREGACION	AGCCACGACC	GICAGCOGG	rendensiten
1000	CTCATCTACC	-	GCGFCCTCGF	GTCCCTGCAC	מכונו ונוצאבנו	CONTROL	GOCAGCAGTT	тесетветов	CAGICOCCC	AGACCACACT
11501	CACCACTACATA		CGACAGEAGE	GICCIGGATT	TOGGACACATAC	TRECARCECG	TTTCCCCACC	TTCGCCCCAG	CCTCCCCACA	ATCTTTANA
10001			GCTGTCGTCG	CAGGACCTAA	ACCCTCCC.TC	ACCCTTICGCC	NANCOCCITYIG	AAGCGGGGTC	CGACCCCTCT	TACANATIT
,		_	888458888			CACATOTTICGE	Tricristat	TCCCCTTAGT	ATGCGGCGC	COCCGATGTA
13601	AMARAMARA		WALL TO A COLOR				BAACAACATA	ACCCCCANTCA	TACGCCGCGC	GCCGCTACAT
		COTACTACGE	THEFT	-		C I Concrete				
13701	TGAGGAAGGT	cereciteeer	CCTACCIACING	TOTOGICAGE	_	אמיינטנינשינ	Christophicz	CCCTTCOATG	CICCCION	CCCCCCOTO
	ACTCCTTCCA	GCAGGAGGGA	GGATGCTCTC	ACACCACTCG	coccoccatc	ACCOCCOCCO	CGACCCAAGA	GOGAAGCTAC	GAGGGGGALLT	GASCIGGEAN
		Kpri								
13801	gracereces	6	GCCTACCOCG	CHENGNANCA	CCATCCCTTA	CICTGAGTTG	GCACCCCTAT	TOTAL		
))	CACCCACCCC	CCATGGACGC	COGATOCCCC	CCCTCTTIOT	CCTANGGCANT	GACACTCAAC	CCTCCCCGATA	ACCTGTGGTG	CCCACACATO	
11901	ACANGTCAAC	GGATGTGGCA	TECCTIONACT	ACCAGAACGA	CCACACCAAC	THETGACEA	CGGTCATTCA	AAACAATGAC	TACAGCCCGG	-
	TOTTCAGITIC	_	NOCCACTICA	TRESTUDEN	GETETEGETTO	ANAGACTOCT	CCCAGTAAGT	THEFTACIO	ATGICOGOCC	
14001	CACACAGACC	ATCANTCT10	ACGACCGGTC	GCACTORGE	CKICCACCTERA	NANCCATCCT	CCATACCAAC	ATGCCAAATG	TOVACCAGIT	-
	graterated	•	TECTEGECAG	ו משפערברנס	CCCICTICGACT	TTTCGTACKIA	CGTATGGTTG	TACOGITTIAC	ACTITICATICAA	Ξ.
14101	ANTARCETTA		GATOCTOTOG	COCTHOCCTA	CTAAGGACAA	TUACITICAG	CTYTAAATACG	AGTGGGTGGA	GTTCACGCTG	-
•	TTATTCAAAT	_		: OCCANCICAT	GATTICCTUTT	AGTCCACT TC	GACTITIATIC	TCACCCACCT	CAAGTGCGAC	GOSCHCCCGIF
				•	Paul			•		
14201	Acresoration	GACCATEACC	ATAGACCTTA		TOMCANCIC GATCHTGGAG	CACTACTTCA	AAGTGGGCAG	ACAGNACOOG	_	
10041	TOBITCHCO				CTAGE ACCITE	CITCATCAACIT	TTCACCCGTC	TOTOTTOCCC	CAAGACCITT	COCTUTABLE
14101	Carre & Activity			" GONTITUAL	CCCURCACTO	CHETTICHENT	CCCTVACACTA	TATACAAACG	ANGCCTTCCA	•
1071	CCATTTCAAA	_			: הכנכרתה היאה	CACAACAGTA	CGGACCCCAT	ATATOTATOC	TTCCCAAGGT	ACCITATIONS
14401		CACCATGCGG	1 GGTGGACTTC	: ACCCACACAC	: מככיריה אהכיאא	CTTCTTCCC	ATCITCANAGE	COCARCCCTT	CCAGGAGGGC	_
	TAAAACG	_			COGACTECTT	GANCANCCCG	TAGACCITTCG	CCCTTCCCAA	GGTCCTCCCG	. ANATOCTAGE

GCCCACTGC CCCCACTCTT TCCCATTCTT TCCCATTCTTT TCCCATTCTTTT GAGAATCCTT CTCTTCGAA	GCAGI-TGITI'A CGTCGACCAT	CTACTICATICA GATGACCAACA CACTICCAAGA GTGAGGTTCT Asid	TTTT	AGTCCAGCUA TCAGGTCGUT				GCACCARTCC	COCCACCATTY GCCCCOOTAA	
CANCAGGGGG CTRATCCGG TGAACGATCA ACTGCTAGT ACCCGAGGTC	ACCCAGINCE GCAGINGRIPS TGGGICATGG CGTCGACANI	CCCAGCAGCT GCCTCCTCCA OFFICCCCCTC CAACGGGCAC	GAGAACCAGA			ANGCISCTUCE TTCGCGAGGC CCATCGACGC		CCCCCTCCAC		GGCGGGGGG AAATCAAAG TTTTAGTTTC
AGATGACACC TCTACTOTGG GTGGAGGACA CACCTCCTGT CCCCTACCGCA GGCGACGCGA	CAGCACCITIC ACCCAGINGC GICGIGGAAG TGGGICATGG	Accretesect TOSACCCGA GCGCCGAGCT CGCGGCTCGA	TCGCTTTCCC AGCGAAAGGG	CTGCGCAACA	COCAGGATAG	COCOCCOOTIC GCCCCCOOTIC GTCGATGACO		CCTOCITIAAC		CCGTGCGCAC GCCACGCGTG GTCCAAGCGC CAGGTTCGCG
	TAAGCAATGA	TECTIONCOTA AGGICATOTA CEGGINGOTAG GGCCACCACC	ACTICITICAA TCGCTTTCCC GAGAACCAGA TGCACAAGGG CTCTTGGTCT	GACGCTANCG CTGCGCACA CTYCGATCGC GACGCGTTGT		AGATGTTTGG TCTACAAACC GCGCACCACC		CCCCCCCCCC		CTGCGCGTGC GACGCGCACG ACGAAGCTAT TGCTTCGATA
* .		TGCTTTYGAG ACGAAACGTG CAGCAACTTT GTCGTTGAAA	TCTCTGACCC AGAGACTGGG	CAGATCACGG GTCTAGTGCC	CCTGGGCNFA	TTCCCANGCA AAGGGTTCCT GCCGCACTGG	COCCOTONC COCCATICAG CCGGTAAGTC	CICCIAACIACIA		CCANTORCOS CCANTORCOS CCANTORCOS CCCCCCCCOS
	GAAAGGEAGT TACAACGTAA CTITGCGTCA ATVITGGATT	TCATTGACCC AGTACCTTGG CGCGCCAGAT GCGCGCTCA		CCTOCTCTCA	THINCANGGE	CCCOGACGG CCCCOGACGCG	GACTURACGC CACTUCACGC GTCACCTGCG	CCCCTCACTCCC		TCAGGGTCG CAGAGGCAAC GTGTATTYKG TGCGGGACTC INGTCCCCAGC GTCCCCGTTG CACATANCCC ACGCGCTGAAAAACTAACTAA GGGGCTGAAAAACTAACTAA TGCAGGGGGGGGTTGAATGAAT AGGTCGCGGGGGTTGAATGAAT AGGTCGCCGG
CCGCACTICITY GRANGINGRAC GGCCTTCTACACTTCT CCTACTTGT CCTACTTGT TO CCTACTTGT TO CAACTTGTCTCT AAGGTTGTCTCT AAGGTTGTCTCT AAGGTTGTCTCT AAGGTTGTAAACTTCTCTCTCTCTCTCTCTCTCTCTCTCT		CCCHARCEC GCCTTACCC TTCCCCTCA	AACTCATCOS CCAGTITACC TTGAGTAGGC GGTCAAATGG	TRAMANGOTT CCTRICTICAL ACTITITION GRACGAGAGT	TUCCCCTACG ACCCCCTACG	ACACAGGETG TGTGTCCGAC	GACCCCGCGC CCAGTGTCA GGFCACAAGGT	CCCCCCACC	GACCACACACA	CHARGEARE GRETATIVATO GEOCCEGITIS CACATANICE GACTOGERICE GATOTATUTA CEGAGCATOR CACATACAT
PRIANCET CETANCATTC O ACTICICA CCATIVITANO C PACCATO GENICAGICA OTCOTOAC COTOSCIAGO OCCACAGO GCOTOAGAGA	CCCCTGACAG	ACCCTCACAC TOGOAGTCTO CCCCGTGACC OGGGCACTGO	GTCTACTCCC	CCACCGTCAG	ACCCCCCCACC TCCCCCCTCC	CCCAGCAATA	TOGCOCCOCO CACOCCOCCOC GTGCTGCTCOT	COTCHCCACC	CTCGAAGGCT GAGCTTCCGA	CACCCCGITY GTCCCCGITY GACTCGTACT CTGAGCATGA
	SCACTAGITY O			CCCACCATCA	CTCACCCCAG	CCTTATATCG	CGCGGGCACT GCGCCCGTGA ACTACACGCC TGATGTGCGG	OCCCCTAGCA CCCCCATCGT	SIII ATGCCGGCCG TACGCCCGGC	CTCAGGGTCG GAGTCCCAGC AAACTACTTA TTTGATGATAT
CCTACGATGA TCTGGAGGGT GGATGCTACT, NGACCTCGCA AGGIGGCAGC AACAGCAGTG TCCGCCGTCG TTGTCGTCAC GGCGAGACCT TTGCGACACG CCGCTGTGGA AACGGTGTGC	AGANGANACE GGTGATCANA CCCCTTACAG ANTACAGAA TCTTCTTTGG CCACTAGITT GGGGACTGTC TCCTGTCTTT	NPM CCFFGCATAC AACTACGGGG GGAACGTATG TTGATGCCGG FTGCCAGACA TGATGCAAGA AACGGTCTGT ACTACGTTCT	TCTACAA	CCCOCCAGC	CACTGOTAAT		AGTGGGCGTG TCACGCGCAC GAGGCGCGCAC	GACCCCCCTC	ACGGGCGGCC TGCCCGCCGG	ACTOCTATGA C TCCCAAGAM A AACCTTCTTT T
14501 C 0 14601 A 14701 C	14801	15001	15101	15201	15301	15401	15501	15701	15801	15901

Figure 15J

		Bgli	_							
16101	CCAGGICATC	900000		בכבפאניאני ניאניאנינאנים		ATTACAAGCT			ANARGANANA GNANGATGAT	CANAGATICAT
	GOTCCAGTAG		AGATACCGGG	GGCTTCTTC		TAATKITTERI GRETTTEGAT		THUGCCCAGT	mrcmm	CTTTCTACT.
16201	GATGATGAAC	TTCACCACGA	CONTRAMENO	CTCCACCCTA	ביומכאבמבדא מינדרהימבאה מבמארקיוחדא		CACTOGAMG		AAAACGTGFT	LICECTACEE.
	CFACTACTIO	AACTGCTGCT	CCACCTTCAC	GACCITCICAT	GACGTOCAT CANCOCCATO COCTOCCOAT		GICACCTTIC	CAGCTGCCCA	TTTTGCACAA	AACOCTOR:
16301	GCACCACCOT	AGECTITACO	CCCCGTFCAGC	GCTCCACCCG	CACCITACAAG		ATCACCINITA		GACCTGCTTG	ACCAGGCCAA
	COTOGTOCCA	TCAGANATGC	REGUCACTUS	CGACGTTGGG	CTCCATGTTC	GCTCACATAC	TACTCCACAT	OCCOCTOCTIC	CTGGACGAAC	TCCTCCGGTT
16401	COARCCCCTC	COCCACTITG	CCTACGGNAA		GACATGCTVG	המודייתנית ההאכפתהאכ	ההאכשתכיאכ		CTAGCCTAAA	GCCCGTAACA
	GCTCGCGGAG	CCCCTCAAAC	CCATCCCTTT	COCCGEATTC	CTGTACGACC	GCAACOGCGA	CCICCICCC	Non-Sort	GAILGEALL	Kml
	Pell				,			The second secon	CATALOGUE ACTURES	Ashering Action
16501	GACGACCACC	NGC COCCOCC	CCAACOTOGC	ACCCAMCAMA ACCCTTCTTT	AGGCTTCTTT TCGCGCCG3A	לייניינים וארניינים איניינים א	AGACCACTGA	ACCORDGOTO	GCACOTCOAC	TACCAMMOT
16601	Managera		GICTIGGMA	MATGACCGT	GGAACCTTAX	CHAGARACECE	AGGICCGCGT	GCGGCCAATC	ANGCAGGTOD	COCCEOGACT
	TCGCGGTCGC	-	CAGAACCTTT	TTTACTGGCA	CCTTRGACTC	GACCTCGGGC	TCCANGCGCA	COCCOOPTAG	TICGICCACC	GCCCCCTGA
16701	GOCCOTOCAG	ACCOTOGACO	TTCAGATACC	CACTACCAGE	AGCACCACTA	TYGCCACCGC	CACAGAGGGC	ATCCACACAC	ANACOTOCOC	GGTTGCCTCA
	CCCGCACGTC	: TOSCACCTOC	ANGICTATOG	CTCATOCITCA	TCGTGGTCAT	MACGGTGGCG	Grencitees	TACCTCTGTG	TTTGCAGGGG	CCAACOGAGT
16801	acadroaca	ATOCCOCOOF	GCAGGCGGTC	DCTMCGGCCG	CGTCCAAGAC	CTCTACOGAG	GTESCHANCES	ACCCGTGGAT	GTTTCGCGTT	TCAGCCCCCC
	CUCCACCOCC	TACOGCOCCA	COTCCGCCNG	CGACGCCGGC	CCACTTCTG	GAGATACCTC	CACGITITIACC	TOCCCACCTA	CAMAGECECIM	ACTEGGGGGG
16901	000000000	COSTICORGO	MAGTACGGCG	CCCCCAGCGC	OCTACTOCCC	CANTATGCCC	TACATOCTTC	CATTIGCGCCT	ACCCCCCGGCT	ATCGTCGCT
	CCGCGGGCGC	: GOCAAGCTCC	THEATGCCCC	GOCCONCCCC	CCATICACCOG	CTTATACGGG		GTAACGCGGA	TCCCCCCCA	TAGCACCGAT
17001	CACCTACCGC	CCCAGAAGAC	GAGCAACTAC	CCCACGCCGA	ACCACCACTG	CAACCCCCCC		CETCGCCAGC	CCGTGCTTGC	CCCGAITICC
	GTCCATCCCC	3 GOGTCTTC1G	CTCGTTGATG	GGCTGCGGCT	TGGTGGTGAC	CTTGGGGGGG	CCCCCCACAGCG	GCAGCGGTCG	GCCACGACCG	GGCCTNAAGG
17101	GTGCGCAGGG	TOCCTCGCGA	ACCARGEON	ACCURACTOC	TRECONCINE	CCCTACCAC	CCCAGCATCG	TTTAAAAGCC	generations	GENCINGEAG
	CACOCOTOCC		Techeconee	TOCCACCACG	ACCIONTIGACG	COCCOATGGTG	COCTCCTAGE	AAATTTTCGG	CCAGAAACAC	CAAGAACCT
										lud/s:
17201	ATAMAGET	r caceroceoe	CICCOTTICC	כאכבושכבשב בובכבודובב בסכובככבשם	ATTCCGAGGA	AGANTGCACC	AGANTGCACC GTAGGAGGGG	CATGGCCGGC		COCCUARCAT
	TATACCGGGA	GTGGACGGCG	GAGGCAAAGG	GCCACOCCCC	TANCHICTCCT		אכידיאכניותם כאינכידככככ	GTACCOGCCG	GTGCCGGACT	GCCCGCCGTA
	Ž.				Sphil					
17301	GCONCOTOCG		CHICACCCCCC	CACCACCOGO GOCGOCOCO GTOGCACCOT	CCX:ATCCCCC	CCCCTATCCT	CCOCTATCCT GCCCCTCCTT			
	COCNOCACOC		CCCCCCCCCCCC	CCGCCGCGC CAGCGTGGCA	OCCITACGCGC	CUCATACIA	CCCCCCACCAA	TAAGGTGACT	AGCGGCGCCG	CTAACCCCC
17401	GRANDINGSAA	A THECATCOOL		GCCCAGAGAC	GECCTINICAD GCGCAGAGAC ACTICATTAAA	MCMGTTTC	ATCTCCTCAAAA	ATCAMATA		
1	CACCOCACT			COCONCICIO	TGACTAATIT	THEFTENACE	TACACCTITIT	TAGITITATI	TTTCAGACCT	GAGAGTGCGA
										Ecoffly
17501	CAS-Melia inter	CHARACTER THE AND THE TREATHER CANGACATER ACTITISCUSE TERROCECTE CONCACTOR CICCOCCUSTS CANGADARC	THETAGAATE	GAAGACATCA	ACTITIOCGIC	TCTGCCCCC	CCACACCCCT	CGCGCCCGTT	CATGGGAAAC	
4	GCGNACCAG	GCGAACCAGO ACATTGATAA AACATCTTAC CTTCTGTAGT	AACATCTTAC	CTTCTGTAGT	TCAAACGCAG	TCANACGENG AGACETATORIC	GCTGTGCCGA	GCTGTGCCGA GCGCGGCCAA GTACCCTTTG	GTACCCTTTG	ACCGITICITAL

Figure 15K

	EcoffV									
17501	ACCAG	CAATATGAGC	GGTGGCGCCT	TEAGGTGGG	Carrentence 1					מכשמישעשט
1	۲	GITTATACTCG	CCACCCCCCC	AGTEGACTEC	פאהנימאניארד: ז	TY GCCGTAAT 1	TITINANCC	AAGCTOCCAA	-	CGICGITCL
1011			AGATGCTGAG	CCATAAGTTO	AAAGAGAGAAA J	ATTRICANCA !	ANACCITICATA	GATCCCCTCG		TAGGGGGT
10//1	Character				_	TAMAGGTTGT 1	TTTCCACCAT	CTACCGGACC	CCACACCCTA	ATCGCCCCN:
					f firefill					
17801	GTGGACCTGG	CCAACCAGGC	AGTGCANAIT	AAGATTAAGA	GTANT:TTGA		CCCTTAGAGG		CCCCTCCA	ACAGTGTCT.
	CACCTGGACC		TCACGITFITA	TTCTAATTGT	CATTENANCT	ACKINGING TO	CANCATCTIC		CCGGCACCIC	TOTCACAGIT
17901	CAGAGGGGG	TOCCCAAAAG	CGFCCCCCCC	CCGACAGGGA	AGAMCTOTO		TAGACGAGGC		GAGGAGGCAC	TANAGCAN
70617	GICICCCCGC	ACCGCTITITIC	GCAGGCGCGG	CCCTCTCCCT	TCTTTGAGAC	CACTGCGTTT	ATCTOCTOGG		crecreers	ATTICGITIC
18001	CCTOCCCACC	ACCCOTCCCA	TCCCCCCAT	CCCTACCCGA			COTAACGCTG	GACCTICCCTC	CCCCCCCGA	CACCACACA
	COACCOCATOO	TOGGCAGGT	AGCCCCCCTA	AGCCCCGGTA . CCGATYCCCT	כעכנועכניכנופ	TCGTGTGTGG	GCATTGCGAC	CTGGACGGAG	COCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	emedicel.
							1		Section 100	
18101	AAACCTGTGC	TOCCAGGCCC	GACCICCOTT	GTTGFACCC			כפניכפכפיכפ	CCAGCIAGICC	CCCATCGFAG	מעשטטטעשנ
	TTTOGACACG	ACCIONACCOCO	CTGGCGGCAA	CNACATIVAGE			200202020	GOLGELAGO	TOC I MOCHAE	CHARLES THE
18201	CCAGTOOCAA	CTOCCAAAGC	ACACTGAACA	CCATCGTGGG	reresests	CAATCCCTGA	ACCCCGACG	ATGCTTCTOA	TACCONTICCO	CACCATACA :
	GGTCACCGTT	_	TOTOACTIGE	COTAGCACCC	אטאטכנכנענ	GITTACCIGNCT	Tegeogeniae	TACGRAGACT	אורניאווערא	Carcalan
10101	TV: BY BY BY BY BY	_	CGCCGCCAGA	CHACTECTS	ACCCCCCCCC	CICCCICCTTT	CCAAGATYGC	TACCCCTICO	ATGATGCCGC	AGIOGICITIA
10501	ACAGTACATA	_	_	CCTCGACGAC	TECHEGRAGE	GCCCCCCCCAAA	CGTTCTACCG	ATGGGGAAGC	TACTACGOCG	TCM-CAGA.
				CERCUITIACI	CCCGGGCTGG	TOCASTITUC	CCGCGCCACC	GAGACOTACT	TCAGCCTGAA	TANCAAGTIT
18401	CATGCACATC	TCGGGCCAGG			CACCACACA	ACCITCAAACG	GCCGCGGTGG	CTCTGCATGA	AGTCGGACTT	ATTIGITICAM
	GIACGIOIAG	•				CCCTTTGACG	CTREGRETICA	TCCCTGTGGA	CCOTOAGGAT	ACTCCCTACT
18501	AGAAACCCCA	_	TACCATCAC			CGCAMCTIC	GACGCCAAGT	AGGGACACCT	OCCACTCCTA	TOACGCATGA
	TCTTTGGGGT	_	•		•	ATGGCTTCCA	CGTACTTICA	CATCCOCKOC	GTOCTGOACA	GOODCCCTN.
18601	COTACAAOGC	•	CTAGCTCTGG			TACCGAAGGT	GCATGAAACT	GTAGGCGCCG	CACCACCTGT	CCCCCCCCAT
•	GCATGLTCCG	CUCCAMPINE		_	CCC.AAGGGTG	CCCCAAATCC	TYCCGAATGG	DATCAACCTO	CTACTCCTCT	TGAAATAAA
18701	TTTTAGGCCC	•	_	-	CACCITICATION	GREGITTAGE	MCGCTTACC	CTACTTCGAC	GATCACGAGA	ACTITATITY:
. 0000	Deadle State		_	_	ACCIANCETEA	GUNGUNYAAA	ACTEACRITAT	TTCCCCAGGC	OCCUMATICA	CCTATAAATA
70887	CINGMOMO	• •	_		-	CGTCGTTTTT	TYPACTOCAT'A	AACCCGTCCG	CCCAATAAGA	CCATATTIAL
-	TTACABACTA		ATAGGTOTCG	AAGGICAAAG	ACCTANATAT	GCCCATAAAA	CATTICACC	TGAACCTCAA	ATACCACAT	CTCAGTGGTA
10681	A BANCHARIA C. F.	_		-	TGGATTTATA	CCCCTATITI	GINNAGITICAG	ACTIGGAGIT	TATCCICTA	GAGIT, MILLAI
10001	CURRACAGAA		-	NGTCCTANAA	-	CAATGAMCC	A'TH'TACGGT	•	AACCCACAAA	TOMANATOR
10061	GCTTIGICIT		_	TCAGGATTTT	TTCTGATCGG	GITTACITTEG	TACAATGCCA	-	TIGGGIGITI	W. I. I. I. W. C.
19101	GORCAAGGCA	•	GCANCANAT	GGANAGCTAG	ANACTICAACT	CHANATCICAA	TITITICICA	-	AGCCGCAGGC	TANCOLONI .
i 1	CCCGTTCCGT	_	carrerrry.	CCTTTCGATC		CCTTTACGIT	MAMAGAGT	-	PLCC-COLORGO	_
19201	ACTIVIDACTICC	-	-	AAGATGTAGA	TATAGAAACC	CCACACACTC	TATAAAGAAT	GTACCCCTCA	TANTECTIC	
	TGAACTGAGG	3 ATTTCACCAT	" MCATGICAC							

Figure 15L

GOSTANTATATS CCCATTATATA	TCCATTGGTS	S AACTTICCAAA	ACCATOTOTA	AACATAGCO .	3 TOGCTCCC():	S CCTGCGCTAC C GGACGCGATA	G GGCCCGAGTA	A AGTITICATA!		C COCORPORATOR				A CTANATACAA T GATTTATGTT	A CCCTGCTAM.	C TCCAGTAACT
ACAACAGCAC TOTTOTCGTG	PPTCCIFICATE AAACGAACTA	ACTCALAGATO TGACTTCTAC	GOGANANGA	CCTUTACTCC	ANGCGAGITUG TTCGCTCACC	CCAATGCTUG	CCTTCTCCTO	COCTOCATTA	ACCACCAGTE TECTGOTCAG	CCGCCGAAAG	CCCTACCTAG	CCCCCAACGA CCCCCAACGA CCCCCTTGCT	Thactatahe	GTCCATCATA CACCTACTAT	GACAGGCCTA	: CATENTALIA
CTAATCTATT GATTACATAA	CATACCAGET	ANATCATCGA TTTAGTACCT	GAAAATGGAT	GCAGAAATTT CCTCTTTAAA	CTACATGAAC	AACCACCACC TTGGTGGTGG	TTANANACCE	CCAACTGCCT	AACGACACCA TTGCTGTGG	CCCGCAACTO	ACCCAGATAT	CCCCTGCTTA	TITACGATCG	CCGTCAGGTO	TACGCGCTTC	
TITTATTCET	ACAGAGCTPTT TOTCTCGAAA	CTTAATAACT	AACARGTEAG TTGTCCAGTC	CCCANCCTUT	ACACCTACGA TGTCGATGCT	CAACCCATT	THETTHEECA	ATGACCTAAG	CATGCTTAGA GTACGAATCT	TCCATCCCCT AGGTAGGGGA	ACACCTACTC TGTGGATGAG	TCCCAATCAC ACCGTTACTG	TTCCTTGTAC AAGGACCATO	AGCCCATGAG TCGCGTACTC	75	BANCARA C
TTATECCTOTT	AGACAGAAAAC TCTGTGTCTTTG	CCACATEITTA GGTCTACAAT	TAMARICTAA	CANTCTAAAT	GATAACCCAA	TCTACAACGT ACCTGTTGCA	מתבידכאפאאפ כסכאפידכידידכ	TCCCTAGGNA	CGCTTGAGGC	COTGCCCATA GCACGGGTAT	CACCCTTAIT	PCARCTUREC AGTERACEDS	CAMAGACITAS	AGAMACTTCC TCTTTGMAGG	TTGGCTACCT NACCGNTGGA	
TACATTIGGTT	TAGATTTGCA	CACKTATIGAT	CTTACCAMA	CCATCERAAAT	AAAAATTTCT	CTTGACTATA	ACATECAGET TOTAGETCCA	Psd mmmm TCTVF7AGAGC AGACCTTCTCG	ACT:GCCTCCA TGXCCA:AGGT	ACTICATACCAA TCACCATGGTT	CTCCCCCCTAC	GACTCTTCTO CTGAGAGAC	CATTGTACTO	CTCCTTCTTT	TCTCSCATTTS AGACCTAAAC	
CAGGCCTAAT 1			PACAGAGACT O	NATAATTTTG TTATTAAAAC	CHECANOGE	ACGCTCGTCC TOCGACCAGG	GIGCCCTTCC CACGGGAAGG	TTAACATOGT AATTGTACCA	GCCCACAAC	ATACCCGCCA	CATCACTGGG GTAGTGACCC	CATTACCTTT	GTTGCCCAGT	ACCCCATGTA TOGCGTACAT	ACACAACAAC	
CTATRICTICAN C		ATCHCGAATC I	CACACTANTY (MGAGITEGA	NAGTACAGTC (ACCTTGGARGE FGGAACCTCG	TOGTCGCTAT ACCAGCGATA	AGGAAGGATG TCCTTCCTAC	TCTTCCCCAT AGAAGGGTTA	GCTCTACCCT	ANGRIMANCEC	AGANGGTIGGE TETTICEACEG	GGGTTACAAC CCCAATGTTG	AGCTACAAGG TCGATGTTCC	TCCTACACCA AGGATGTGGT	
CCCCACAT C			CCACTGGGAG C	AAAATGAAAT J	CCHOTTCGAT	ACCATACATTA	TOCTOOCCAA	OTGGAACTIC CACCTIGAAG	TACCCCACCT	CCCCCAACAT	CCTTANGACT	CACACCTITTA	TTGACGGGA	TATCCCAGAG	CAGOTOGGCA	
ACAACTAATE E	8 8	2 2	F 3	T.Y.	N S		CCCTCAATGT	ACACCTACGA TGTGGATGCT	CATTIGCETT	TATCTCTCC	CCTTCACGC	TTACCTCAAC	AAGCGCTCAG TTCGCGAGTC	AGGGCTTCTA	CCTGATGGT	
19301	19401	19501	19601	19701	19801	19901	20001	20101	20201	20301	20401	20501	20601	20701	20801	

Figure ISM

									Marrana Marran	1
21001	TTATOTCCAT		ACAGACCTAG	GCCANANCCT	TCTCTACGIC	אאנידאנינונגינ	ACCCCCTIACA		CAGGTURATE	CCATOGACGA
	AATACAGGTA	CCCCCCTGAG	TOTCTOGACC	COSTITIOGA	AGAGATGCTRE	THEMETICANS	TOCOCCANICT	CTACTCAAAA	CTCCACCTAG	GGTACCTGCT
21101	OCCCACCCTT	CTITAIGIT	TOTTICANGE	CITTIGACGIG	מאנכמאנוני	ACTIANTORIA	والمرازوم المالا	ATCGNAACCG	TGTACCTGCG	כשכמככבנו.
	COGGICAA	GAAATACAAA	ACAMCTICA	GAMCTGGAC	CAGGGACACA	TUGICISCUT	CHECHECOCYC	TAGCTTTGGC	ACT "TICACOC	GTGCCGGAV 1
										Ligiti
21201	Tresconsch	ACCCACACA	ATAMAGAAGC	AAGCAACATC	AACAACAGCT	GCCCCCATOG	GCTCCAGTGA	GCAGGAACTG	AAAOCCATTO	TCAMAGATOT
	Adecedecear	recoercing	TATTICTICG	TTCGTTGTAG	TIGITICITOIA	COURGETACE	CGAGGICACT	CONCUMBAC	TTTCOGTAAC	AGTTICTAGA
21301	recorrected	CCATATETT	TOCGCACCTA	THACAAGCGC	TTTCCAGGCT	THUTTETEC	ACACAAGCTC	GCCTGCGCCA	TAGTCANTAC	COCCIGENCY .
	ACCAACACCC	GGTATAAAA	ACCCCTGGAT	ACTGTTCGCG	AAAGGTCCCA	AACAAAGAGG	TGTWTTCGAG	COGACGCOGT	ATCAGITTATO	CCGGCCAGIX
21401	GAGACTOGGG	GCOTACACTO	GATGGCCTTT	GCCTOGAACC	CGCACTCAAA	MCATOCTAC	CTCTTTGAGC	CCTTTCGCTT	TTCTCACCAG	COACTCAADC
! !	CTCTGACCCC	CGCATGTGAC	CTACCGGAAA	COGACCTTOR	CCCTCACTIT	TINTACCATE	GAGAMCTCG	GGAAACCGAA	AAGACTOGTC	GCTGAGTTC
21501	AGGITTACCA	GTTTOAGTAC	GAGTEACTEC	TCCCCCCTAG	CGCCATTCCT	Terreceed	ACCOCTOTAL	AACGCTGGAA	ANGTECACCC	AAAGCGTAC .
	TCCAAATGGT	CAMACTCATO	CTCAGTGAGG	ACCCCCCCATC	GCGGTNACGA	AGAAGGGGG	TOCCGACATA	TTGCGACCTT	Trcaggrada	TTTCGCATOT
21601	GOOGCCCAAC	TOGGCCGCCT	GTGGACTATT	CTGCTGCATG	TTTCTCCACG	CCTTTGCCAA	CTGGCCCCA	ACTCCCATGG	ATCACAACCC	CACCATGAN'
	CCCCOOCITIO	ACCCOCCOCA	CACCTGATAA	GACGACGTAC	ANGNOGTEC	GCAAACGGTT	GACCGGGGTT	TCACOGTACC	TAGTGTTGGG	OTGGTACTT .
		Kpre					•			
21701	CITATIACES	GOOTACCCAA	CTCCATGCTC	AACAGTCCCC	ACCTACACK	CACCCTGCGF	COCANCCAGO	AACAGCTCFA	CAGCITICCTO	GAGCGCCAC"
1	GAATAATGGC	CCCATOOOTT	GAGGTACGAG	TTGTCAGGG	TCCATCTCCC	GTCCCACGCA	GCGPTGGTCC	TYGYCGAGAT	GTCGAAGGAC	CTCGCGGTR
21801	COCCTACT	CCCCAGCCAC	AGTECECAGA	TTAGGAGCGC	CACTICITITE	TOTCACTTOA	ANAACATGTA	AAAATAATOT	ACTAGAGACA	CTTTCAATAA
	GCGGGATGAA	GOCOTICOGTG	TCACGCGTCT	AATCCTCGCG	Grewann	ACAGTGAACT	THITIGIACAT	TTTTATTACA	TOATCICTOT	GAAAGTTAT""
21981	ACCETABATIC	TITTATTION	ACACTETEGG	GIGATTATT	ACCCCCACCC	TRICCGTCTG	COCCUTTIVA	ANTCAMOD	GOTTICTICCCO	COCATCBCTA
	TCCOTTTACO	MANTANACA	TGTGAGAGCC		TOCTOCTGGG	NACGCCAGAC	GCGCCAAATT	TITAGITICC	CCAAGACOGC	GCGTAGCGA 'r
22001	TOCOCCACTO	GCAGGGGACAC	GITGCGATAC	TOGTOTTAG	TYCTCCACTT	NANCTICAGGG	ACAACCATCC	GCGGCAGCTC	OCTOMACTIT	TCACTCCACA
	ACCCCGTCAC	COTCCCTGTG	CAACGCTATG	ACCACAMATE	ACTINGGITGAN	TTTGAGTCCG	TGTTGGTAGG	CCCCGTCCAG	CCACTTCANA	ACTCAGGTGT
					EcoffV					
22101	GOCTGCGCAC	CATCACCAAC	GCCTTTACCA	GGTCGGCCCC	CCATATCTEG	AAGTCGCAGT	TORGGCCTCC	000000000	CCCCAOTTOC	GATACACAGG
	CCGACGCGTG	GTACTOGTTG	CGCANATCGT	ככשפטבכפכפ	CICTATAGAAC	TTCAGCGTCA	ACCCCGGGGG	COCCACCCCC	GCGCTCAACO	CTATISTICS.
22201	GTTGCAGCAC	TOGANCACTA	TCAGCGCCCG	GTGGTGCACG	CHARCEAGCA	COCTUTATION	CCAGATCAGA	TCCGCGTCCA	CONCONCOC	GTTGCTCAGO
	CAACGTCGTG	ACCTIGICAT	AGTCGCGGCC	CACCACGTCC	GACCAGTCGT	GCCACACAG	CCTCTAGTCT	AGGCGCAGGT	CCAGGAGGCG	CAACGAGTCC
22301	CCCAACCCAAC	TCAACTITIOG	TAGCTGCCTT	CCCANANAGG	מכניניניושלינינ	ACACICITITICAG	TTGCACTCGC	ACCUTAGIOO	CATCAAAAGG	TGACCGTGCC
	COCTIGOCTIC	AGTTGAAACC	ATCCACCCAA	COGNITITICS	מפכניכיוכנינים	TCCGAMACTC	AACGTGAGCG	TOCCATCACC	GTAGTTTTCC	ACTROCACOO
22401	CONTETENCE	GTTAGGATAC	AGCCCCTCCA	TAMAGCCTT	GATCTGCTTA	AAAGCCACCF	CAGCCTTTGC	GCCTTCAGAG	AAGAACATGC	CGCNAGACTT
	OCCAGACCC	CAATCCTATG	TCCCGGACGT	ATTITICGGAA	CTAGACGAAT	TITICGGTGGA	CTCGGAAACO	COUNK! I: TC	TICTIONACG	· "ITCTCAA
			SE!		•		IIA)			
22501	CHASABAR	TGATTGGCCG	מאכאמטכנפנ		CACCACCTTG	GREGINGAGO CAGGACCITIC CONCRETE	CGAGATY TTG	ACCACATTTC	GCCCCCACCG	ACCACATTIC GGCCCCACCG GITCTICACG
* * * * * * * * * * * * * * * * * * * *	CGCCTITIG		CTGTCCGGCG		CAGCACGTUIC GTCGTGGAAC	CICAGCICACAA	CCTCTAGACO	TECTETAAAG	ccacecruec	CCCCCCTCCC CANGAAGTGC

Figur 15N

CONCECCT ATTENENTA ANGUITECTT GCACGAGGAA TAAATAGTAT TACGAAGGAA 1581	ANCOTACHO GICACCICTO CAANGARTA TACGAACATC CAGTGGAGAC GITTYICTKIN'		GGTACTTGTC CATCAGCGGG CACACAGCCT CCATGAACAG GTAGTCGCGC GCGCGTCGC 1	TCTTCCTCTT	PICATTAGCA CCOGIGGOTT GCTGAAAGGG AACTAATCOT GGCCACCCAA CGACTTTG-7:	AGAAGGGCGC TCTTCCCGCG	CTCAGAAGGA	TOGITICOCCO NCCANCCCCC	GATCATOGAG	CACCTTCCCC GTCAAGGCAC CCCCGCTTGA GTGGAAGGGG CAGCTCCTTG GGGGCGAACT		ACAGAGSATA AAAMALPAKA ULAMAALOW IGICTCCTAT TTTTCGTTCT GGTCCTGTTS Pall	TTTTCTTCT PS GAAGCATCTG CTTCCTAGAC	TTTTCTTCT PARAGCATCT GAAGCATCT CTTCGTAGAC CTGCTATTCT GTGGATAAGA	THTTCCTTCT BAACCATCT CACCTATTCT CACCTATTCT GREGATAGA TGCTTSCCAC ACGAACCOTC	TTTTCCTTCT PARACCATCT CACCTATTCT GTGGATARGA TGCTTGCCTC ACGARCGOTO	THE TOTAL THE TO	TTTTCCTTCT PARACCATCT GAACCATCT CTCCTAGAC CACCTATTCT TGCTTGCCAC ACGAACGOTG CGCTCTCATA GCGACACTATA CGCTCTCATA CGCTCATA CGC
ATTICAATCA CGTOR TAAAGITAGI GCACO	TOCKETKETO ATOC ACCKEARCAC TACE	CAGCITATAAC CCGO GICGACGITIG GGCG	TTATCCACCT GGTA AATAGGTGCA CCAT	CACTITICCOC TICO	THICKCATIC TICA	CCCCCCCCA CCC	GCACCAGOGO OTOT COTOGTOGOGO CAGA	COGOCCIOCO ACO	FICHCETATA GOC	ACGCGCCTAC CAC	CTCACTACCA ACA	GAGTCATOOT 10TH	•	•				
CCACTCTACG ATT	מהמהאמככרה ד ספ בהרמדכיאמכ אכפי	TRATICANATT CAG ACCACTTOTA GTO	CTTTAGATCG TTN GAATCTAGC AAT	ACCETANTIT CAC TESCATTANA GTE	CONTRACTICE THE	CICTICATOR GROGARIA GROGARIA GROGARIA CCO	משיחומכפרם פכא ככאכאכפרפכ פסי	GCGACGGGGA COC CACTGCCCCT GCC	GRECATTICE THE	GATGCCGCCA ACC		TOCTCCTVAGC GA						
CONTRICTOR OF GENEVALORS	נאתנאומאכ פט מאנאומאוט כט	CACAACAACG AC	TRANGTTRES CO	CGGGTTCATT: AC GCCCAAGTAG TI	recaetratee of occasions	CCACCATTAC C	CCCCCCCTC G	CONCECCION O	CTTCCCGACT G	בהתאתיתים ב	AGCGAAGACG A					CCCTACCICA C CCCTACCICA C CCCCTCAC C CCCCTCAC C CCCCCCAC A		CCCATCACA CCCATCACA CCCCATCAC CCCATCACC CCCATCACC CCCATCACC CCCATCACC ACCCCACAC ACCCCACACC ACCCCACACCCACACC ACCCCACACCAC
ממשמעלעים ה במכשמטאמעט מ	CCCTCCCCAC G	CCTCACAAAG G	CCCTCATCA A	GCACACTCAG C	ATTCARCCOC (TCCTCXCTGT C	ACCAGCTACC O	معودودحددو و	COCTOCTCCT	TCCCCACCAC	ACCEPTIFICATA		-			GGGGACGAM CCCCTGCTTT GCACGATGT CGTCGCTACA CCTGCCCAAC	GGGGGGGAN CCCCTGCTTT GCACCGATGT CGTCGCTACA CGACCCCACC GCTCGGGTTG	GGGGACGAM CCCCTGCTTT GCACGATOT CGTCGCTACA CCACGCCCAC GCACGCCCAC GCTCGGGTTG TCCCTGTGCCA ACCACGTT
כירירידינאמכי (פאנוזיאאניזעט (TCGATETENG (GCCCCATCAT CCCCATCAT		} ∪ ∪	OCTCGTCTTC CCAGCAGAAG	THETETHTET	TCCOCCGCCG AGGCGGCGCC	GCTTTTTTCG CGANNAACC	CCACCAAAGC	CCCTCTGAGT	ACCACCACTC		7					
TOCTAGACTO	AAGCTCGCCT TTTCGAGCGGA PSt1	TOCAGGRATC ACOTECTING		CHCCCACGCA	CCCCCACTO	GCGCCACATC	ANTOGCCAAA	COCCTCATCC	COCOCTCOO	CCTAACCGCC	-		-					
ATCTTROCCT 1 TAGAACCOGA A	SACACTT	CAGGTACGCC		CCATGCCCTT		ACCATTIGIA	TCTTGGGCGC	CTCCATACGC	CCACCOCOTC	AGAAGGACAG	CCTCCTCCTT		GCAGAGGCAA	GCAGAGGCAA CGCCCCGTT GCGCCATTAT	GCACACGCA CUTCTCCGTT GCGCCATTAT CGCGGTAATA ACCCCCCAA	GCMGAGGCAA CGTCTCCGTT GCGCCATTAT CGCGTAATA ACCCCCAAA TGGGGGGTTT	GCACAGGCA CSTCCCSTT GCGCGSTANTA ACCCCCCAAA TGGGGGGTTT TTTTTCCAAA	GCGGGGTAT CGCGCCATTAT GCGCCTATAT GCGCCCAAA TGGGGGGTTT TTTTTCCAAA
22601	22701	22801	22901	23001	23101	23201	23301	23401	23501	23601	23701		23601	23801	23901 23901 24001	23901	23901 24001 24101	23901 24001

Figure 150

PMRRAdSgag MER682

24201	CCTCGCTCAA	CGAAGTUCCA	MANATCTITG	ARGGITTITES TCCCAGAAGC	ACGCGACGAG	AMGREGATARIO	CANACCICTUT	CCTTGTCCTT	MCAGCGANA	atchacter Tactetercy!
24301	CHCTGGAGTG	TTCGTCCAAC	TCCAGGGTGA	CAACGCGCCCC	CTAGCCGTAC	TANANCTICAG	CATCGAGGTC	ACCCACTITIO	CCTACCCOOC	ACTTAACCTA
24401	CCCCCCAAOO		AGTEATGAGT TEAGTACTEA	GACCTGATEC					AGNACANACA	GAGGAGGG""
24501	TACCCGCAGT	TOCCGACGAG	CAGCTAGGGC GTCGATCGCG	GCTCGCTTCA	AACGCCCCCAC	CCTCCCACT	TGGAGGAGCG ACCTCCTCGC	ACCCAAACTA	ATGATGGCCG TACTACCGGC	CAGTHICTECT GTCACGAGGA
24601	TACCGTGGAG	EB	TGCAGCGGTT ACGTCGCCAA	CTTTGCTGAG	CCGGACATGC GGCCTCTACG	AGCGCAMGCT	AGACCIANACA	TTGCACTACA	CCTTTTCGACA	GOGCTACGTA CCCGATCCAT
24701	COCCAGGCCT	GCAAGATCTC COTTCTAGAG	CAACCTGGAG	CTCTGCAACC	TRGTCTCCTA ACCAGAGGAT	CCTTGGAATT	TTGCACGAAA	ACCOCCTTOO OCMAACGTO TOOCOGAACC COTTTTGCAC		CTTCATTCCA
24801	CGCTCAAGGG GCGAGTTCCC	CCAGGC	עש	TCCGCGACTG	CCTTTACTTA	TTTCTATGCT ACACCTGGCA		GACGECEATO GOCOTITICAE CTGCCGGTAC CCGCAAACCG		AGCAGT3CT1' TCGTCACGAA
24901	CCTCCTCACG	AACCTCAAGG	AGETCCAGAA	ACTGCTAAAG TGACGATTTC	CHAMACTICA	AGMACCTATG TCCTGGATAC	GACCICCTTC CTCCCGCAAG	ACCAGCCT	CCCTOGCCCC	OCACCTORCY: COTOGRACCO!:
25001	GACATCATTT	ACCCCCAACG	CCTCCTTANA	ACCCTF3CAAC TKGGACGTTG	AGCCAGACGG	AGACTICACC	ACTCANAGCA TCACTTTCGT	TOTTGCAGAA	CTTTAGGAAC	PTTATCCTN . AAATAGGATU
25101	AGCGCTCAGG TCGCGAGTCC	ANTETTOCCC	GCCACCTOCT CGCTGGACGA	GTGCACTTCC CACGTGAAGG	TACCCTGAAA	GTGCCCAFTA	AGTACCGCGA TCATGGCGCT	ATGCCCTCCG	CCGCTTTGGG	GCCACTGCTA
25201	CCTTCTGCAG	CCTTCTGCAG CTAGCCAACT GGAAGACGTC GATCGGTTGA	ACCTIGGCTA	CCACTCTGAC GGTGAGACTG	ntnategnag Taitaccttc	ACGTGAGGGG TOACGGTCTA TGCACTCGCC ACTGCCAGAT Kml		CTGGAGTGTC GACCTCACAG Pstl	ACTGTCGCTG TGACAGCGAC	CAACCTATRIC
25301	ACCECECENCE	CGAGGGACCA	TTGCAATTCG AACGTTAAGC	CACCTCCTTA	ACGANAGICA	AATTATCGGT	ACCTTTGAGC TOGAAACTCG	TECAGGETICE	CTCGCCTGAC	GANNAGTECA CTTTTCAGGT
25401	CGGCTCCGGG	GITGANACTC	ACTCCGGGGC TGAGGCCCCG	TOTOGACOTO ACACCTGCAG	CCGAATGGAA	CCCAAATTIG	TACCTGAGGA	CTACCACGCC	CACGAGATTA	GGITCTACGA
25501	AGACCAATCC TCTOGTTAGO	COCCCOCCTA	ATGCGGAGCT TACGCCTCGA	TACCACCTAC	GTCATTACCC CANTAATGGG	ACTURE OF THE PROPERTY OF THE	TCTTGGCCAA	TTGCNAGCCA	TCAACAAAGC AGTTGTTTCG	CCCCCANGA GCCCTTCTT
25601	ANAGACGATG	GAMAGOGIACO CTTTCCCTGC	GOOGGITTTAC CCCCCAAATG	TTGGACCCCC AACCTGGGGG	AGTCCGGCGTCA TCAGCCCCCT	OCACCTCAAC CCTCGAGTTG	CCAATCCCC	CGCCGCCGCGCA	GCCCTATCAG	CHICAGCCA

Figure 15P

PMRKACISgag MER682

Pell

				TOOTTOOOT						
25701	GGCCCTTGC	TYCCCACOAT	CCCACCCANA	AAGAAGCTGC	ALT TRACTIC	שגענגנענם נ	מאכטאטטאטטאט	ANTACTOCICA (CAGTCAGGCA	CAGGACACTET
•	CCCGGGAACG	AAGGGTCCTA	CCGTGGGFTT	THETHEGAGG	Try: Actions	CAN'T TO CONTINUE (רויקכורכנוננ	TTATGACCET (OTCACTCCGT	CHCCTCCN .
						Herelill				
25801	TUCACGAGGA	CCACCACCAC	ATCIATCICANG	ACTORROBAG	נינידאהאהינאה	ÇÇ	AGGICGNAGA	GETGTCAGAC (GAACACCGT	CACCITION .
	ACCTGCTCCT	cercercero	TACTACCTTC	TGACCCTCTC	GGATICTGCTK;	CTTCGAAGGC '	TCCAGCTTCT	CCACAGACAG	CTTTGTGGCA	GTGGGAGC+'A
25901	CGCATTCCCC	TCGCCCGCCC	CCCAGNANTC	GCCAACCGGF	TECAGGATEG	CTACAACCTC	CGCTCCTCAG	0000000000	CACTGCCCGT	TOTOTOGACCC
	GCCTAAGGGG		GOTTETTAG	CCCTTGCCCA	AGCITECTACE	GATICITUGGAG	CCGAGGAGTC	כעכמסמממכה	GTCACGGGCA	ACCOCATGGG
26001	AACCGTAGAT	GGGACACCAC	TGGAACCAGG	GCCCGTANGE	CCANGCAGCC	CCCCCCTTTA	GCCCANGAGC	NACAACAGCG	CCAAGCCTAC	COCICATOR.
	THOOCATCTA	cccrcrccra	ACCITIGGICC	CGGCCATTCA	GGTTCGTCGG	CUGCUUCANT	CGGGTTCTCG	TIGHTOTICGE	GGTTCCGATG	OCCANGTACCU;
26101	GCOCOCACAA	GAACGCCATA	grtactract	TGCAAGACTG	TESCHERECAAC	ATCTCCTTCG	CCCGCCGCTT	1CTTCTCTAC	CATCACGGCG	ACCION.LANCION
	COCCOROTT	CFTCCCCTAT	CHACGAACGA	ACCTACTGAC	ACCCCCGTTG	TAGAGGAAGC	GCCCCCCCAA	AGNUNANGATO	GTAGTOCCGC	ACCITICAACG
26201	CCGTAACATC	CINSCAPTACT	ACCOTCATCT	CTACAGCCCA	TACTRICACER	GCCCACAGAGGG	CAGGNACAGG	-	CAGAAGCAAA	מאכנועכנסנוע
	OCCAPICTAG	GACCTANTGA	TOCCAGTAGA	GATGTCGGGT	ATGACGTGCC	COCCGICCCC	Greensred	reaccoorer	arcriconn	ccacroaccr
26301	TAGCAAGACT	CTGACAAAGC	CCAAGAAATC	CACAGCGGCG	GCARCAGCAG	GACCACCACC	GCTOCGTCTG	GCGCCCAACG	AACCCGTATC	CACCCGCGAG
	ATCOTICTGA	GACTOTITICG	OGTTCTTTAG	Grencecee	ceresteere	CICCICCICG	CGACGCAGAC	cocosornoc	TTGGCCATAG	CTGGGCGCTC
26401	CTTAGAACA	GUATETETICS	CACTUTATAT	CCTATATITIC	AACAGAGCAG	GROCCANGAA	CANGACCTOA	AAATAAAAA	CADOTETETO	CGATCCCTCA
	GAATCTTTGT		GTGAGACATA	CCATATAMG	TRINCTOTIC	CCCGGTTCTT	GITCTCGACT	THATHUM	GICCAGAGAC	CCTACOCACT
26501	CCCGCAGCTG	CCTOTATCAC	ANAGCCAAG	ATCAGCITICG	GCGCACACTG	CHACACGCGG	AGGCTCTCTT	CAGTAAATAC	TOCOCCACTOR	CTCTTAAGGA
	GCGCGTCGAC	GCACATAGTO	trrnc6ctrc	TAGTCCAAGC	CCCCTCCCAC	CTICIOCOCC	TCCGAGAGAA	GICATTIATO	ACCCCCCACT	GAGAATTCC .
26601	CTAOPTICGC	GCCCTTTCTC	AAATTTAAGC	CCCANANCTA	CGTCATCTCC	ANCONCCACA	CCCCCCCCCA	GCACCTGTTG	TCAGCGCCAT	TATORGCAAG
	GATCAAAGCG	COOCANAGAG	TITAMATICG	COCTITION	OCNUTAGAGG	TOCOCCOGNOT	CACCCCCCCGGT	CCTCGACAAC	Aprecedora	ATACTCGTTC
26701	GAMATTCCCA	COCCCTACAT	OTCGAGTTAC	CACCCACANA	TOCKACTTOC	GOCTGGAGCT	GCCCAAGACT	ACTCAACCCG	AATAAACTAC	ATGARCGCGG
	CITTAAGGGF	OCCOGNATIONA .	CACCTCAATG	Greenstr	ACCCTGAACG	CCGACCTCGA	CCCCTTCTCA	TCACTTGGGC	Tratttgatg	TACTCGCGCC
		Ecoffy			m see	Ecolii				
26801	GACCCCACAT	_	GTCAACGGAA	TACOCOCCCA	-	CCCDANCCCA ATTITUTCETOR	NACAGGCGGC	TATTACCACC	ACACCTCOPA	ATAACCTTAA
	CTGGGGTGTA	_	CAGTIGCCTT	ATGCGCGGGT	CRACITITIOGCT	TANGAGGACC	THETECGECG	ATANTGGTGG	TOTOGAGCAT	TATTCCAATT
26901	TCCCCGFAGF	TOCCCOCTG	CCCTCGTGTA	CCAGGAAAGT	CCCCCTCCCA	CCACTGTGGT	ACTITICATION	GACGCCCAGO	CCGAACTTCA	GATCHCTANC.
	AGGGGCATCA	ACCOORCGAC	GGGACCACAT	GGTCCTTTCA	CATOCCIACCACT	GUTTOACACCA	TOMGGGTCT	CTGCGGGTCC	GGCTTCAAGT	CTACTGATTO
27001	TCAGGGGGGG	AGCTTGCGGG	COCCUINCE	CACAGOGING	SCHOOL COOK	GCAGGGTATA	ACTICACCTICA	CAATCAGAGG	GCGAGGTATT	CAGCTCAACT
	AGTCCCCCCC	•	GCCGANAGCA	OTOTOCCACG	CCYUCGGIAC	CCTCCCATAT	TGAGTGGACT	GITAGICICC	CGCTCCATAA	GICGACTICA.
27101	ACCAGNCOCT	GAGCTCCTCG	CTROCTCTCC	GTCCGGACGG	GACATTICAG	ATCGGCGGCG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TICATTICACG	CCTCGTCAGG	CANTCCTAAC
	TUCTCAGCCA	CTCGAGGAGC	GARCCAGAGG	CAGGCCTGCC	CTCTAAAGIC	TAGCCACCAC	GCCCGCCGAG	AAGTAAGTGC	GCAGCAGTCC	GTTACCATTC
	Pall									
27201	TCTGCAGACC	: repreciens	ACCOURTE				CASAGTTTGTG		ACTITIANCCC	CHICHCOOCA
	AGACGTCTOG	3 AGCAGGAGAC	TCCCCCCGAG	S ACCTCCGTAA	CCTTGAGACC	TTAAATAACT	CCTCANACAC	CCTACCCACA	TCAMATTGGG	GANGAGCCCT

Figure 1501

PMRKAd5gag MER682

GCAGACCAAC COTCTCCTTT ATATCCAGT TATAGCTCTT GCACAGGG :A CCTGTCCCTT AGAATTAATT	ATCTCTCC TACAGAGGE TCCTTACCT TAGTTACCT TGTTTACCAG ACAMATGGT TGTTTACCAG	GATTAGAT 1. GCCTGCT1 1 CGGACGACAC GTCAGCCCAC	ACENCAGN TGGTGTCT13 TTACAGTT1 F AATGTCAAAA	GTOJCCCCCA CACCGIGGOT TACANARICA ATGITTAC ' ATGITTAN 'T TYGITTAN 'T
ANDTICOROUNG TITCACCITCTC CCCCACCITCTC GGGCTCCTAG TAGTTCACCC ATCACTCGC TAGTAAATAC ATTATTATG	TACTTTTAAC ATGANAATTG AACACCACC TTOTOCTGGG TCAATAACTC AGTTATTGAG	ANDOCTOBOC TYCCOAGCG TYCCCAGCGG AGTGGAACG	TATAAAATGC ATATTTTACO QAGTATAATG CTCATATTAC	AGTATAAGT TCATATTCAA CTATATTAAA GATATAAATT GACGAACGT TCTATGTGGG
ACTGAATGTT TTACTTACAA CTTTGAATTG GAAACTTAAC CCCCCCTGC CCCCCCTGC GCGGGGGGCG GTGCTGAGTA CACGACTCAT CACGACTCAT	CCTTACCTOG GGAATGCACC CATCAGAAA GTAGTCTTTT CGCACAGACC GCCTGTCTOG		CCACCACTCT CCTCGTGAGA TCACACTACA CTCTGTGATGT	TACHGEANG TACHCECTRET CATGGGATGA CATGGGATGA CGAAATGAGC CGAAATGAGC
GACASCTACG CTTACTTATGG ATTTAGGAT GATTAGGGG CAAATGGGTC TGGCATCTCT ACGGATGGA	CCAMPRICAN GGTTCCGCTT TCAGCTACTC AGTCGATTTTTC TCTGAAAAAG	GATTATANA CAAATACTTA ATACTAACGC TATGATTACG GATGATTAAG	GCTANTIAGT CGATTACTCA CGATTACTCA CCGTCGGTCC	TACCATGRACE OCTITIONET CGAAACCAGA ACCACTAACT TACTGATTGATT TACTGATTGATTGATTGATT TACTGATTGATTGATTGATTGATTGATTGATTGATTGATT
CCTCAGCCTC CCTCAGCCTC CACTCCGGTG CTCAGCCAC TCATCGGGA ACTAGCCCT TGILL TGILL ACATCTTGT	CCCANGCANA GOOTTCOTTT CTCTCCGAGC GAGAGGCTCG ACCGTANACC TCGCATTTOG	CTACACACA GATCACACC CTTTATTCTT GAANTAAGAA AGATGATTAAG	CGCAGCTGAA GCGTCGACTT TATGCTATTT ATACGATAAA	ACACACTOTA TACAGTGCTC TACAGTGCTC ATGTCACGAG A AGCTAATATCA TCGATTACAG TCGATTACAG
ACGCGGTAAA CTTTTGCCGG GAAAA GAGG GGCCGTAGGC GGCCGTAGGC GATTAGATCA GATTAGATCA	AGANGTORGE AGANGTORGE ACTACIACITO TGCTCTCTTG CTACCGCCTG GATGGCGGAC	MANAGEREAG TTTCCCCCTC TTCTGATTCT AACACTAAGA TCGCCACCA AGCGTGGGT	ATGITACATT TACAATGITA GTATGITGIT	TTTTATGANA AVANTACTET CTATACTANT GATACGATTA TANGTTACAA I ATTCANTSTT CGTCANTSTT CGTCANTSTT CGTCANTSTT CGTCANTSTT
CCTAACTTRS GGATTGAAAG GCCACAAATA CGGTGTTCAC GGGAAACTT CCCTCTCGAA CCTTTCGAA CCTTTCGAA	AACGCCACCG TTGCGGTGGC GAGTGAGTCT CTCACTCAGA TYCACCCACAC	GTATTAGGCC CATAATCCGG ATTCTCTGTC TAAGAGACAG AACGCTGGGG	ING CCAGCCTGTA TTC GGTCGGACAT CA ANATTGGCAA UT TTTAACCGTT BS11071	TACTITICCA ATACTOCACTO TACTOCACTO ACGACOTORC CTTATITIVO CATATATOCOCOC
TCANTTANT AGTTANATA CACTGTCGC GTGACAGCGG TTACCGCCCA AATGGCGGGTT TTGCAACTGT AACGTTGACA	CCATCCTGTA GGTNGGACAT AACCCAGACG TTGGGTCTGC CACCGGCCGC GTGGCCGCG	ANCECTTAGG TTGGGAATCE COTTGGGGTT ECAACCCCAA CAGCTTTTTA	TTTTAACGAO AAAATTCCTC CACAAAACA GTGTTTTTTGT BSI	CTTTATETO GAAATTICAT TGGCACTTTC ACCGTANAG 1 AAGAANTGG 1 TTCTTTACG 1 GAATAGATT
ACTATECOSA TGATAGOCCT TGTGGACCAG GOCGTCCGGC CCGCAGGCCG	GCTCCTATCG CGAGGATAGC CAACAGTTC GTTGTCAAAG ACGAGGGGT TGTCAAAG	CTCGAATCTT Xbal CTCGAATCTT CTCGAATCGG GATCTTAGCC CATTTATTGT GTAAATAACA	AAAAGTGGA TFFTCCACCT GCFTATTCCC CGAATAAGCG	ACTCATAGA TCACTATTT TCACACACCA ACCTTTTCACA TATTCACACATA ATARAACTCCTT
CCTCCCGGC GGAGGCCGG TGCGCTGAA ACGCGGACTT CCCGGGGACTT CCCGGGGCG GGGCCGCGTG	ATATACTOS TATATORCC CTOTOSTTA GACACTAAAT CCGGGAACUT GGCCTTGCA	TECHCETECA TECHCETA TECHCETTECT AGTECAAAGA ACTICTAAAGA	GOTACCACC CCATGGTGGG ATGAAAAGCT TACTTTTCGA	CCAGGGTMAA GGTCCCATTT CCAAATTTGTG GTTTTTGTGTC CTCCGTCGAA AAAGGTTGGC TTTTCCATTGG
27301 27401 27501 27601	27701	28001	28301	28501 28601 28701 28801

Figure 1SR

. 28901	CATATACAAC	CTTK3AAGTCA	GGCTTCCTGG	CHUBARICA GRETICOTIFG ANGICAREAT CHOACHTING CEAGEACCTG TCCCGCRAT TIGHTCCAGT CCAACTAGAG CGAACCACAC	CHICACTTRIGG	CCAGCACCTG '	PCCCGCGGAT 1	PROFITCEAGT O	CAACTACAG	מאכככאכייכ
		GAACTTCAGT	CLEMENTAL	GAACTTCAGT CLEADICHAITE THEAGTESTA GACTGAANGE GGTEGTFINE ARGGGGCCTA ANCANGGTCA GGTTGATGTE GETHAGTHI GALLAGTHA	CACTGAAACC	GGTCGTTGTAC	AGGCCCCTA 1	ANCANGETCA O	SCHICATICE (SCHOOL STORY
29001			ACCAACGCGG	GACCAACACA ACCAARGGGG CEGECGGTAC CROACTAACA ATTACACACA ATTACACE ACTITETACT THUISTAATA ACTIGAATAA ACTICAATAA AC	CCICACTTACA	TUTACCACAA	ATACACCCCA	ACTITICIDEC 1	PPTOTCAATA A	ACTYSCIANTAA RAACCCTANT
,			ומווואוו	וא איז אפרניא זוי	CALL MANAGER	· COLONIA COLO		CERTIFICATION	and and a	ACCACCCATE
29101	_	rogractica	CCATAGCGCT	CCATAGCGCT TATTTTTTATA TCACCTATATA TTATCHER F CALCAC CINCACCA PROCESSION	TCACTTAITA	דועוניוואייו ד	באוני זויכוסר	The second of th		WOOD WATER
	GAACCCGTAC	ACCACCANGA	CCTATCCCCA	ACCACCANGA GGTATGGCGA ATACAMAGAT ACGAMTANT MITACAKAGA GARANCAGA GALILOGOGI HOGGGGGA	ACGGAATAAT	ANTACACT UN	いっかんしていてい	197971118	10000000	
29201	TATAGTCCCA	TCATTGTGCT	ACACCCAMC	TCATTOTGCT ACACCCCANC ANTIATYINA TETATOGAT GARCCGAPTO ANACACATOT TCTTTCTCT TACAGTATGA	TYCATAGATT	משענמניורות	ANACACACATOT	TCTTTTCTCT	FACAGTATGA	TTANATCACA
	ATATCAGGGT	_	rerecentric	AGTACACCA TOTOGETTIG TIACTACET AGGIATOTAA CCTOCCTCAC TITOTOTACA AGAAAGAGA ATGICATACF AATTIACTCT	AGGTATCTAA	CCTGCCTCAC	THETETACA	ACAAAAGAGA 1	ATGLCATACT	AATTTACTCT
		Xhoi								
29301	CATGAITICCT	CCAGTTTTTA	TATTACTGAC	CONGISTISTA TATTACTICAC CCTIVITICG CTTTTTICHS CONSCICCAC ATTOOCIACA OFFICECACA FCOANGIACA CTOCATIC A	CTITATION	CONSCINCANC	AFFOGCTOCO	GIFFICTCACA	PCGAAGTAGA .	CTGCATTC A
ì	GTACTAAGGA	GETCAMANT ATANTGACTG GGAACAACGC GAANANGAG GGACGARGTTG TYACGGAGGC CAAAGAGTGT AGCTTCATCT GACGTAAA.F.	ATAATGACTG	CCANCANCOC	CHANNANCAC	CCACCACICTYS	1'MCCGACGC	CAAAGAGTGT	AGCIFICATOR	CACOTANO .T.
					Pstl	Pstl				
29401	GCCTTCACAG		TTACGGAITT	REPAINTMENT THACAGAITH GTCACCCTCA CACTCATCTA CACCATCATC ACTOTICATCA TCCCCTTTAT CCAGTGCATT GACTGGGTAT	CACTCATCTO	CACCCACATO	ACTIONOGRICA	TCCCCTTTAT	CCAGTGCATT	GACTOGGIV:T
			AATGCCTAAA	AGATAAACGA AATGCCTAAA CAGTGGGAGT GCGAGTAGAC GTCGAAGTAG TGACACGAGT AGCGGAAATA GGTCAGGTAA CTGACCCAAA	GCGAGTAGAC	GTCGGAGTAG	TGACACCAGT	AGCCGGAATA	GCTCACCTRA	CTGACCCAGA
							Ecofi	_*		
29501	The state of the s	T Transfer	AGACACCATC	HYPARANTE AGACACCATC CCCAGTACAG GGACAGACT ATACCTGAGC TICTTAGAAT TCTTTAATTA TGAAATTTAC TGTGACTTT	GGACAGGACT	ATAGCTGAGC	TTCTTAGAAT	TCTTTAATTA	TOWATTIAC	TGTGACTT"
3	CACACGCGA		TCTGTGGTAG	ACCIVITATEGE TOTATECTAG GOOTCATOTE CETATECTE ANTEGACTED ANGMATETA AGMANTIMAT ACTITIMANTO ACACTOMAN	CCTGTCCTGA	TATCGACTCG	ANGNATCTTA	AGAAATTAAT	ACTITIAAATO	ACACTOMAN
20601			ATCTGGGTT	THEREACET ARTICULAR TOTACCEGA CETECANGE TEAANGACAT ATATEATGEA GATTEACTE TATATGGAAT ATACCAGIT	CCTCCAAGCC	TCAAAGACAT	ATATCATGCA	GATTCACTCG	TATATOGAAT	ATTCCAACIT
``````````````````````````````````````	GACGACTAA		TAGACGCANA	AANCOTOGOA TAGACCCAAA ACAAGGGGCT GGAGGTICCG AGTITCTGTA TATAGTACGT CTAAGTGAG ATATACCTTA	GGAGGTTCGG	AGTITICITA	TATAGTACGT	CTAAGTGAGC	ATATACCTTA	TAAGGTTCAA
							Pstl			
29701	L OCTACAATGA	AAAAAGCGAT	CTTTCCGAAG	ANAMAGGAT CTITCCOANG CCFGCTIATA TCCAATCAFC TCTGTIATGG TGTTCTGCAG TACCATCTTA GCCCTAGCTA TATATCCC+A	TCCAATCATC	TCTGTTATGG	TUTTICTICAG	TACCATCITA	GCCCTAGCTA	TATATCCCIA
		PTITTCGCTA	GAAAGGCTTC	TITITICECTA GAAAGOCTIC GGACCAATAT ACGITAGIAG AGACAATACC ACAAGACGIC ATGGIAGAAT CGGGATCGAT ATAINKKAAT	ACCITAGTAG	AGACAATACC	ACAAGACGTC	ATGGTAGAAT	COOGATCGAT	ATATACKGAT
29801	CCTTGACAT	r cocrociaco	CANTAGATES	OCCIDIANCO CAATAGATIC CATGAACCAC CCAACITITIC CCGCGCCGG TATGCTTCCA CTGCAACAAG TIGITIGCGG CGGCTTTGT.	CCAACITICC	ככפכפכבנפכ	TATOCTTCCA	CTCCAACAAG	TTOTTOCCOG	COCCUTION
	COAACTGTA		GTTATCTACG	CTACTTGGTG	CESTICANGO	DOUBLES	ATACGAAGGT	GACGITIOTIC	AACAACGGCC	GCCCAAACA 1
		•							Xbal	- <b>*</b>

CHCTOCACCC TTATTANGAC CCHGTGCGGT CTCAAAGATC TTATTCCGTT TAACTAATAA AAAAAAATA TATTTGTAGT GAATGAATTT TAGTCAATCG GAGACGTGGG AATAATTCTG GGACACGCCA GAGTTTCTAA AATAAGGGAA ATTGATTATT TTTTTTATT ATTTCGTAGT GAATGAATTT TAGTCAATCG 30301

GIVENTIONES GROADARGCE CATTACEATA ACTERGENET CHETARANANE CRANGGETGE ATTERCTERE CTTOTEARGG ACCTERINGAT CAGACTEC TOGACTECETA

TOCOGITITEA GIOGRIFICITO TEATTAINEST GOCCIGIGGE GGAATGGATG TICAACGGTT

MOTICEARAGE CACCETACGAC AGENATACCA CCOGACACCG

TRAGGGGGGG TITTAGTCGAT GAAATTAGAT TGTCCTCCTC TACTCACTGT GGGATCTAGA TCTTTACCTG AGACGCAGGG CACCGGCCGA GCAACAGGCC ATGAATCAAG AGCTCCAAGA CATGGTTAAC TTGCACCAGT

CCTGCTAGAA GCACGATCTT CTCCTAAAGC GAGCATTTCG

COTCGGTTAG

**GGAATTATTA**CCTTAATAAT

30001

GCANANGGGG

CGFFFFCCCC

GAANTIGGTO

30201

CCAGCCANTC AGCCTCGCCC

29901

PETECOTOCO GROXCOSCO COTTOTOCO TACTTABITIC TOGRAGITICI GIACCAATIG AACGIGGICA

CCANGCOTY", GGTTC/F/M:T

AAGTTGCCAA

CCTTACCTAC

ACCITICACIC ACCICCACAG ANAICARCTA CITITAATICTA ACARGAGGAG.ATGACTGAGA CCCTAGAACT AGAAAATGGAC

## PMRKAd5gag MER682

10401										
Totos	TTTAAAGACA	GGTCAMTA	GICCICCICCICC	AGGAAGGGA	GCTCCCAGCT	CHOCHATIGO	ACCITICATEC	TOCCHOCAA	CHITCTCCAC	ANTCTARATG
30501	GAATGTCAGT	TACCACTOR		Character	TATCTTOTATE	Thermore	TCAARTGCCC	AACACCCTACT	The Act of the Contract of the	The bronders
	CTTACACTCA					MCMCGTCT	ACT TECHESCO	TICTOGCAGA	CTTCTATIONA	ACTTGGGG A
30601	GTATOCATAT	_	_		delight de la		Transport of the state of the s	De tomanion	TO THE CHARLES	
	CATAGGTATA			•	TTGACACGGA AAAGAATGAG	GAGGGAAACA	TAGEGGGTA	CCCAAAGTTC	TOTAGGGGG	ACCCCATCAC:
				Ē.	-					
30701	TETTTGCGCC	TATCCGACC	TCTAGTTACC	TCCAATGGCA	TCCAATGCCA TGCTTGCGCT	CAAAATCGGC	AACCKSCCCCC	CTCTGGACGA	GGCCGGCAAC	CTTACCTCC1:
	AGAMACGCGG	ATAOGCTTOG	AGATCANTGG	AGGITACCGT	ACCIMICACION	GTITTACCCG	TTGCCCGCAGA	GAGACCTUCT	CCGGCCGTTG	GANTGGAGE 3
30801	AAAATOTAAC		CCACCTCTCA	AANNANCCAA	GTCANACATA	AACCTCGAAA	TATETRICACC	CCTCACAGTT	ACCTCAGAAG	CCCTAACTOF
	TITIACATIO	GTGACACTCG	COTOCACACT	TTTTTTKKITT	CAGTITICIAL	TTCGACCTTT	ATAGACGTUS	CCAGTGTCAA	TOGAGICTIC	GOCATICACA
30901	<b>GOCTOCCOCC</b>	GCACCTCTAA	TGGTCGCGGG	CAACACACTC	ACCATGCAAT	CACARGECEE	GCTAACCGTG	CACGACTCCA	AACTTAGCAT	TOCCACCCAA
	CCGACGGCGG	CGTGGAGATT	ACCAGGGGCC	GTTGTGTGAG	TESTACCITA	GTGTCCGGG	CCATTCCCAC	GTGCTGAGGT	TYGAATCOTA	ACCOTOCC1 "
31001	OGACCCCTCA			GCCCTGCAAA	CATCAGGCC	CCTCACCACC	ACCGATAGCA	GTACCCTTAC	TATCACTOCC	TCACCCCC11"
	CCTOCOCOACT	GICACAGICT	TCCTTFRCGAT	COCCACCTTT	GTACTCCOGG	CCACTOCTOG	TOGCTATOOF	CATGGGAATG	ATACTGACGG	ACTOCOCCA
31101	TAACTACTGC		THOGOCATIG	ACTTGAAAGA	<b>GCCCATTTAT</b>	ACACANANTO	GAMMETAGG	ACTAMAGIAC	DOGGCTCCTT	TOCATGTAL .
	ATTGATGACO	GIGACCATCG	AACCCGTAAC	TOANCITTICT	COCGENANTA	TCTOTTTYAC	CTTTTGATCC	TCATTTCATO	CCCCGAGGAA	ACCEACATTV
31201	AGACGACCTA	ACACITIGA	CCGTAGCAAC	TOGICCAGGIF	GRCACTATTA	ATAATACTTC	CTTGCMACT	AAAGITACIG	GAGCCTTGGG	TITIGATICA
	TCTOCTOGAT	TTCTCAAACT	OCCATCGTTG	ACCAGGTCCA	CACTCATAAT	TATTATGAAG	GAACCITICA	TTTCAATOAC	CTCGGAACCC	NANCTAN .
31301	CAAGGCAATA	TOCAACITAA	TOTACCAGGA	GGACTAAGGA	TRAINCTCA	MACAGACGC	CTTATACTEG	ATOTTACTTA	<b>TCCOTTTGAT</b>	<b>GCTCANAACC</b>
	GTTCCOTTAT	ACCITICAATT	ACATCGTCCT	CCTGATTCCT	AACTAAGAGT	THORENCE	GAATATGAAC	TACANTCAAT	AGGCNAACTA	CONDITITION
31401	AACTAAATCT	ANGACTACCA	CAGOOCCCTC	TTTTTATANA	CYCAGCCCAC	AACTTGGATA	TTAACTACAA	CANAGOCCITY	TACTTOTTTA	CAGCTTCAA
	TTGATTTAGA	-	GTCCCGGGAG	ANAANTATIT	averceeere	TTGAACCTAT	ANTTGATGTT	GTTTCCGGAA	ATGAACAAT	GTCGAAGT71'P
		Fendia			-					
31501	CAATTICCAAA	AAGCTTGAGG		CACTGCCAAG	GOOTTOATOT	TTGACCCTAC	AGCCATAGCC	ATTAATGCAG	DADATOGOCT	TOATTTOY: F
	<b>TTANGGTTT</b>	TTCOAACTCC	AATTCGATTC	GTGACGGFTC	CCCNNCTACA	AACTGCGATG	TCGCTATCGG	TAATTACGTC	CTCTACCCGA	ACTTANACCA
31601	TCACCTAATG			ANAACMMAA	TRACCATO	CCTAGAATTT	GATTICAACA	AGCCTATGGT		CONCRER
	AGTOGATTAC	GIGGITTORO		THEORITH	AACCCGTACC	GGATCTTAAA	CTANCITICS	TCCGATACCA	ACCATTTCAT	CCTTGACCCIU
31701	TTAGTTTTGA	CACCACAGGT	OCCATTACAG	TAGGNAACAA	ANTANTOAT	AAGCTAACTT	Tritograccac	ACCAGCTCCA	TCTCCTAACT	GTAGACTAAA
	AATCAAAACT	GICGIGICCA	CCCTAATOTC	Arcermen	TTTATTACTA	TYCGATTGAA	ACACCTRAGIG	TOGTCGAGGT	AGAGGATTGA	CATCICATIT
31801	TOCAGAGAAA	GATGCTAAAC	TCACTITIOGT	CTTAACAAAA	TOTAXCACTO	AAATACTTGC	TACAGITITICA	OTTITIOGCIA	TTAAAGGCAG	THRACTECA
	ACOTOTOTI	ACOTOTOTOT CTACGATITIG	AGTGAAACCA	GAATTGTTIT	ACACCGTCAG	TTTATGAACG	ATCTCANGE	CANAACCONC	AATTTCCOTC	AAACCGAGGT
31901	ATATCHERAA	ATATCHERA CAGITICAAAG	TOCTCATCTT	ATTATAAGAT	THENCONNA	TOGAGTGCTA	CTANACAATT	CCTTCCTGGA	CCCAGAAATAT	TR. JAACTETA
	TATAGACCIT	TATAGACCIT GICAAGITIC	ACGNETAGAA	TAATATTCTA	AACTCCTTTT	ACCTCACGAT	CATTICITAA	GGAAGGACCT	COCTCTTATA	ACCTICAAAT
	E	gli)								
32001	GARATGGAGA	TCTTACTGAA	GARATOGAGA TETTACTICAA OGCACAGECT		TGFTGGATTT	ATRICCTANCE TATEARCITTA TECAAAATET CACOGTAAAA CTGCCAAAAG	TATCACCTTA	TCCAAAATCT	CACOGTANAA	CTGCCAAAAG
:	CTTTACCTCT	AGNATGACTT	CITTACCICT AGNATGACIT CCGTGTCCGA		ACAACCTAAA	TATGTTTGCG ACAACCTAAA TACGGATTGG ATAGTCGAAT AGGTTTTAGA GTGCCATTTT	ATAGTCGAAT	AGGTTTTAGA	GTGCCATTTT	GACCOTTTTC

Figure 15T

32101	TAACATTGTC	AGTCAAGTTT	ACTTANACCG	AGACAAAACT	AAACCTGTAA	CACTAACCAT	TACACTANAC	GGTACACAGG	ANNCAGGNGA	CACAACTC!A
	ATTICTAACAG	TCAGTTCAAA	TOANTITINGC	אכזוניזדיזויזע	TTWENT	GICATTICCTA	ATGREATITE	CCATGTGTCC	Triorceich	CTGTTRIAC: 71
32201	AGTGCATACT TCACGTATGA	CTATOTCATT GATACAGTAA	TTCATCCGAC ANGTACCCTG	TAXTICTCRCCC	ACAN TACAT TETTVIATVITA	TANTGAMATA	TTTCCCACAT NANCCATICTA	CCTCTTACAC	TTTTTCATAC ANAMGTATG	ATTRECECAN: TAACGRETTY:
32301	AATAAAGAAT	CONTINUEDIN	ATCHTTCAAC	CACAMATAM	TTCAATTICA	GAAAATTTCA	ACACTETETE TEACTEMAN	CATTCAGTAG	TATAGCCCCA	CCACCACATA
32401	CCTTATACAG	ATCACCGTAC	GAATTAGTTT	CTCACACAAC	CCTACTATTC	AACCTERCOAC	CTCCCTCCCA	ACACACAGAG	TACACAGTCC	TTTC: NCCC 11:
32501	GCTOGCCTTA		TATCATGGGT	AACAGACATA	THETTAGETO		CACCGTTINE	TCTCGAGGCA	AACGCTCATC	AGTOATATT
32601	ATAMACTCCC TATTICAGGG		ACTTAAGTTC TGAATTCAAG	ATCHCCTCT TACACCCACA	CCTANATION TO CONTROL		TOCTICTCCAA	CTYGCGGTTG	CTTAACGGGC	GOTTONIONA:
13761						PSI ************************************	www.	PERFERENCE	المدرسلاجات الاعتبار	
10.35	TTCAGGTGCG	-	CATCTCAGTA		GICCTATCCC	CCACCACGA	CONCORCOCO		ACCIACCCCCG	CCCCCACCA
	Patt									-
32801	CCTGCAGGAA	TACAACATOO	CAGTOCICIC	CTCAGCGATG	ATTCCCACCG	CCCCCCACACAT	AAGCCCCTT		CACAGCAGCG	CACCC-TOA!
	GGACGICCIT	ATGITOTACC	GTCACCAGAG	GAGTCGCTAC	TAAGCGTKAGC	CCCCCTCGTA	Trecection	CAGGAGGCCC	GTGTCGTCGC	GTGGGACT**;
			amman .							
32901	TCACTTAAAT	_	ACTOCAGCAC	AGCACCACAA	TATTCTTCAN	ANTECCACAG	TGCAAGGCGC		CCTCATGGCG	COCACCACAG
	AGTGAATTTA	GICCIOICAT	TCACCTCGTG	TCCTCGTCTT	ATANCANGIT	TTACCCTCTC	ACCITICCIACO	ACATAOGTTT	CCMOTACCGC	CCCTCOTCTC
33001	AACCCACOTG	-	CACAAGCGCA	GCTAGATTAA	GTYGGGGACCC	CTCATABACA	COCTGGACAT	ANCATTACC	TCTTTTGGCA	TCTTCTAATT
	TROOTIGCAC	COGTAGTATG	GIGTICGCGT	CCATCTAATT	CACCGCTGGG	GAGTATFTGT	GCGACCTGTA	TITIGTAATEG	MGNAAACCOT	ACAACATTAA
		Kpri								ligid 1
33101	CACCACCTCC		TAMACCICIO		OCCCCATCCA	CCACCATCCT	NACCAGCTO		0000000000	TATACACTA"
	GTGGTGGAGG	OCCATOGTAT	ATTOCAGAC	TAATTIGTAC	CGCGSTAGGT	GOTGOTAGGA	TTICKITCGAC	COCTTTTGGA ExpRV	cocococcco	Atanthreac:
33201	ACCOMACCOS	GACTGGAACA	ATGACAGITAG	AGAGCCCAGG	ACTUALANCE	ATCCATCATC	ATRICTCOTCA	TCATATCAAT	CTTOCCACAA	CACAGGCACA
	TCCCTTOGCC	CTCACCTTGT	TACTGTCACC	reresource	TONOCATTOG	TACCTAGING	TACCAGCAGT	ACTATAGETA	CANCCGROTT	GIVENICGIGI
										Pell
33301	COTOCATACA	_	ATTACARGCT		TACAACCATA	TCCCAGGGAA	CANCCCATTC	CTGNATCAGE	GTAAATCCCA	CACTGCAGGG
	GCACGTATGT	DAAGGAGTCC	TAATICTICGA	GGARGGCGCA	ATCTINGTAT	AGCGTCCCTT	CTTCGCTAAG	GACTTAGTCG	CATTTAGGGT	GIGACCITC:
33401	MGACCTOC	_	CGTTGTGCAT	Tretchanging	TTACATTCCG	מבשנונשנונענונע	ATGATCCTCC	AGTATOGTAG	CCCCCCTTC	TCTCTCANA
	TTCTGGAGCG	TCCATTCACT	<b>GCAACACGTA</b>	ACAGITITCAC	ANTOPAAGCC	מהוכהוכהבכ	TACTAGGAGG	TCATACCATC	GCGCCCAAAG	ACAGAGTETE
33501	CCTCCATCTO	GATCCCTACT	GTACCCIACTO CATCCICAC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ACCCACATEG TGGCTCTAGC	TEST TREESTE OF ACADECA	AGTGTCATCC TCACAGTACG	CANATOGNAC	OCCOGACUTA CGCCTGCAT	CTCATATTT.

Pg/II

33601	CTCAACCAAA	ACCAGGTGCG TOOTCCACGC	GGCGTGACAA	ACAGATETEC	האניאקה הניאם ניאניאקה היים היים היים היים היים היים היים הי	YMGCCCATTA GATCCCTCTG TGTAGTAGTT GTAFTATATC CACTCTCTA AMGGCCAAT CTAACCAGAG ACATCATCAA CATCATATAG GTGAGAGAFF	CATCCCTCTC CTANGE	TCTAGTAGTT ACATCAA	GTACTATATC	CACTCTCTTA GTGAGAGAITI'
33701	AAGCATCCAG		GCTTCGGGTT	CTATGTAAAC	TCCTTCATGE	נטינטינושינינ	ACTATACATE ACTATACT	CACCACCCCA	GANTNAGCCA	CACCCAGCC/ GTCGC:TCGCII
33801	ACCTACACAT TGGATGTGTA			CCCTCCTCCC					AGATTATCCA	AAACCTI JAJA TTTGGAGITT
33901	Bgfil ATGAAGATCT	ATTAAGTGAA	CGCGCTCCCC	TCGGGTGGCG	TEGTENANCT	CTACAGCCAA	AGAACAGATA	ATCCCATTO	TAAGATGTTG	CACANTOGET
34001	TECANAGGE	AAACGCCCT	CACGTCCAAG					TAMACAPTCC ATTITOTANGO	ACCACCTTICA TCGTGGAAGP	ACCATGCCCA TOGTACGGGT
34101	ANTANTECTO			TATCTCTAAG	CAMPECCGA	ATATTAAGTC TATAATTCAG	CCCCCATTGT	AAAAATCTGC TTTTTAGACG	TCCAGAGCGC AGGTCTCGCO	CCTCCACCTT
34201	CAGCCTCAAG			AAITICAGGIT	CCTCACAGAC	CTCTATAAGA	TICANAGED	GAACATTAAC CTTGTAATTG	AAAAATACCO	CGATCCCGTA GCTAGGGCAT
34301	CCAGGGAAGC			CGTGCAGGTC	TGCACOGACC ACGTGCCTGG	ACGIGCCTGG TCGCGCCGGT	CTTCCCCCCC	ACCITOGIAC TCCTTGGTAC	ACAAAAGAAC	CCACACTOAT
34401	TATGACACOC		ATACTCOGAG CTATOCTAAC TATGAGCCTC GAFACGATTG	CAGCGTAGCC	Hindill  CCGATGTAG CTTGTTGCAT  GOCTACATTC GAACAAGGTA		CCCCCCCTA	ATMANATOCA TATTTTACGT	AGGTOCTOCT TCCACGACGA	CAAAAAATC! GTTTTTTAG!
34501	CCCTTTCCGA				GCTCATGCAG CGAGTACGTC	ATAMAGGCAG TATTTCCCFTC	GTAAGCTCCG CATTCGAGGC	GAACCACCAC	AGAAAAAGAC	ACCATITITITIC TGGTANAANG
34601	TCTCAAACAT			ACACAMATA	ANATAACANA	ANANCATITIA TTTTGTAAAT	MCATTAGAA	COONCACATA	CAACAGGAAA	AACAACCC1" TTGTTCCGAA
34701	ATAMOCATAR		GGCCATGCCG	GCGTGACCGT	AAAAAAACTG	GTCACCOTGA	TTANAMGCA AATTTTTCGT	CCACCCACAG	CTCCTCGGTC	ATGTCCGGA9 TNCAGGCCTC
34801	TCATAATOTA	AGACTCOOTA	AACACATCAG TIGIOTAGIC	GTTGATTCAC	ATCOSTCAGT	GCTAANAAGC CGATTTTTCO	GACCGANATA	GCCCGGGGA CGGGCCCCCT	ATACATACCC TATGTATGGG	GEAGACH GTAG CGTCCGCATC
34901	AGACAACATT	ACAGCCCCCA	TAGGAGGTAT	AACAANATTA	ATAGGAGAGA TATECTETET	ANACACATA	AACACCTGAA	AMACCCTCCT	GCCTAGGCAA	AATAGCACCC TTATCGTGGG
35001	TCCCGCTCCA ACOCCGAGGT	-	CTCGCGAAGG	ACAGCGGCAG TGTCGCCGTC	CCATAACAGT		AGTANANAG TCATTTTTTC	AAAACCTATT	AAAAAAACAC TTTTTTTGTG	CACTCGACN: GTGAGCTGTG
35101	CCCTOCTCCA	r CAATCAGTCA	CAGTGTANAA GTCACATTTT	AAGGGCCAAG			GACTAAAAAAA CTGATTTTTT	TGACGTAACG		TOTTTTTO
35201	CCCAGAAAAC	COCACOCOAN B OCOTOCOCTT	CCTACGCCCA	GAAACGAAAG	CCANNANACC	CACAACTTCC	TCAAATCGTC AGTTTAGCAG	ACTICCGITT	TCCCACGITA AGGGTGCAAT	CCTCACTTCC

figure 15V

## pMRKAd5gag MER682

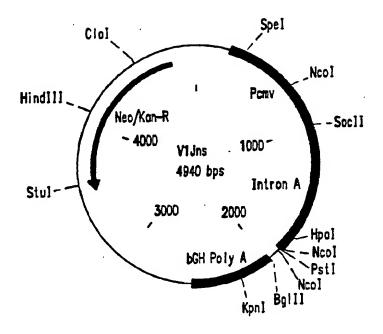
35301	CATTTTAAGA	AAACTACAAT	TCCCAACACA AGGGTTGTGT	TACAAGTTAC	TECHCOCOTAA NGCCHARATT	ANCETACETIC ACCEGECES		TYCCCACGC	CCGCGCCACG	TCACANACTC AGRETTEGAG
•						PR	Fcofil			
35401	CACCCCCTCA	TTATCATATT	GCCTTCAATC	CAMATANGO	TATATTATTG	ATCATCITAA	TTAMBAATTC	GGATCTGCGA CCTAGACGCT	CCCCAGGCTO	GATEGECTY: CTACCGGAAG
35501	CCCATTATOA	THEFTETEGE		ATCGGGATGC	CCGCGTTGCA	COCCATOCTO	TCCAGGCAGG	TAGATCIACGA	CCATCAGGGA	CAGCTTCAAG
35601	OCCAGCAAAA	GGCCAGGAAC	COTABABAGG	CCCCOTTGCT	CCCCANAMG	CATAGGCTCC		CGACCATCAC	ANAMATCOAC	OCTCAAGTC7. CGAGTTCAGT
35701	CAGOTOCCOA	AACCCCACAG			TTTCCCCCTC AAAGAGGGGAC	CANCETCCCT	CONCECUTOR	CCTUTTCCGA	CCCTGCCGCF	TACCCCATAC ATGCCCTATY:
35801	GACAGGCGGA	TICTCCCTTC	CCCTTCGCAC	GCGCTTTCTC	ATARCTCACG	CTCTACGUTAT	CTCANTITOGG	TOTAGGTCOT ACATCCAGCA	TCGCTCCAAG AGCGAGGTTC	CTCOCCTCP.
35901	TOCACGAACC	CCCCGTTCAG	CCCGACCOCT	CCCCCTTATC	CGGTAACTAT	CCACATICAGE	CCAACCCGGF	AAGACACGAC	Trategeeac Aatagegetg	TOOCAGCAGY: ACCGTCGTC
36001	CACTOOTAAC	AGGATTAGCA TCCTAATCGT	GAGCGAGGTA	TOTAGGCGGT ACATCCGCCA	CCATCTCTCA	TETTGANGTO AGAACTTEAG	GTOGCCTAAC	TACCOCTACA	CTAGAAGGAC	AGTATTIGGF TCATAAACCA
36101	ATCTOCGCTC TAGACGCGAG	TOCTGNAGCC ACGACTTCGG	AOTTACCTIC fCAATGGAAG	CCTTTTTCTC	TTCTTAGCTC	TTGATCCGGC AACTAGGCCG	ANCANACCA	CCCCTOOTAG	CCCTCCTTTT	TTTGTTTVGC. AAACAAAGGT
36201	AGCAGCAGAT	TACGCGCAGA	AAAAAAGGAT	CTCAAGAAGA	TCCTTTGATC ACCANACTAG	TTTTCTACGG	GGTCTGACGC	TCAGTOGNAC AGTCACCTTG	GAMMCTCAC	GTTAAGGGAT
36301	<b>TTTGGTCATG</b>	AGATTATCAA	AAAGGATCTT	CACCTAGATO	CTTTTAAATC	ANTCTANAGE	ATATATGAGT TATATACTCA	ANACTIGGIC	TOACAGITAC	CAATCCTTA
36401	TCAGTGAGGC AGTCACTCCG	ACCTATCTCA TOGATAGAGT	CCCTAGACAG	TATTTCGTTC	ATCCATAGTT	GCCTGACTCC CRSACTGAGG	CCGTCGTGTA	GATAACTACG	ATACGGGAGG TATGCCCTCC	GCTTACCATY: CGAATROTAG
36501	TOOCCCCAOT ACCGGGGTCA	GCTOCAATGA	TACCCCCAGA	CCCACOCTCA	CCGGCTCCAG	ATTTATCAGE TAAATAGTCG	AATAAACCAG	CCACCCCCAA	GGCCGAGCG	CAGAAGTGGT
36601	CCTGCAACTT	TATCCOCCTC ATAGGCCGAG	CATCCAGTCT	ATTAATTIGIT TAATTAACAA	CGCCCTTCG	TAGAGTAAGT	AGTTCGCCAG TCAAGCGGTC	TTAATAGTTT	GCCCAACGTT CCCOTTGCAA	GTTCCCATTC
36701	CTACAGGCAT	COTCOTOTCA	CGCTCGTCGT GCGAGCAGTA Pwil	TTOGTATESC AACCATACCG	TTCATTCAGC AAGTAAGTCG	TCCGGTTCCC AGGCCAAGGG	AACGATCAAG TTGCTAGTTC	GCGAGTTACA CGCTCAATGT	TGATCCCCA ACTAGGGGGT	TOTTGTGCAA ACAACACGTT
36801	AAAAGCGGIFT TFFFFCGCCAA	ACCICCTICO TEGAGGAAGE		GTCCTCCGAT COTTGTCAGA CAGGAGGETA GCAACAATCT	AGTANGTING TCATTCAACC	CCCCACTGTT	ATCACTCATG TAGTIGAGTAC	CANTACCOTC	CACTGCATAA	TTCTCTTACT ANGAGAATGA
36901	GTCATGCCAT CAGTACGGTA	CCGTAAGATG	GAANAGACAC	ACTGGTGAGT TGACCACTCA	ACTUMCCAA TGAGTTGGTT	CACTANGACT	GAATAGTGTA	TOCHGCGACC ACGCCGCTGG	GAGTTIGCTCT CTCAACGAGA	TACCCARCAT

Figure 15W

# PMRKAd5gag MER682

CANCACOCO TANTACCOCO CCACATAGA GAACTITAAA AGTACTICATE ATTGAAAAAC GITCTTCGAB GCGAAAACTC TCAAGGATCT TACCACAGT GITGTGCCCT ATTATGGCGC GGTGTATGBT CTIKIAAAFT IYACGAGTAG TAACCITTTB CAAGAAGGCC CGCTITTGAG AGTICCTAGA ATGGGAAAAA	CAAAAACAGG AACGCAAAAT	TCAMBGTTAT TCTCTCATCA	GTCTAAGAAA CCATTATTA: CAGATTCTTT GGTAATAATA	ID NO: 27) ID NO: 28)
GCCAAAACTC	TCTCCCTCAG	GAAGCATTTA CTTCGTAAAT	OCCACCTGAC COCTCGACTG	taat (SEQ atta (SEQ
GTTCTTCGGG	CACCAGGGTT	CAATATTATT	CCCGANANGT	CATGACATTA ACCTATAAAA ATAGGGTAT CACGAGGCCC TTTCGTCTTC AAGAATTGGA TCGGATTCT TAAT (SEQ ID NO: 27) GTACTGTAAT TGGATTTT TATCCGCATA GTGCTCCTGG AAAGAGAAA TTCTTAACCT AGGCTAAAA ATTA (SEQ ID NO: 28)
ATTOCAMAC	CTTTTACTTT	CTTCCTTTTT	CCCTOTATTIC GCGTGTAAAG	Bamfill AAGAATTGGA TUC TTCTTAAGCT AGG
AGTGCTCATC TYTAGGAGTAG	TCTTKINGTATAT AGAAGTGGTA	TACTCATACT	AGGIRITHTER TECECAAGGE	TTTCGTCTTC AAAGCAGAAG
GAACTITAAA	ACTCANCTGA Tracetticact	AAATGTTGAA	ATAMACAMAT TATITIGETETA	CACGACGCCC
CCACATAGEA	CCACTCGTV3C	GCCCNCACCG CCCCNCACCC	ATTAGAAAA TAAATCTTTT	<b>ATAGGGGTAT</b> TATCCGCATA
TANTACCOCO	TCGNTOTAAC AGCTACATTO	AGGGNATAAG	ATTIGAATGT TAAACTTACA	ACCTATAAAA 1038ATATITI
CAACACCOOA	GAGATCCAGT	COGCOTITIT	OCCCATACAT CCCCTATGTA	CATGACATTA
37001	37101	37201	37301	37401

Figure 15X



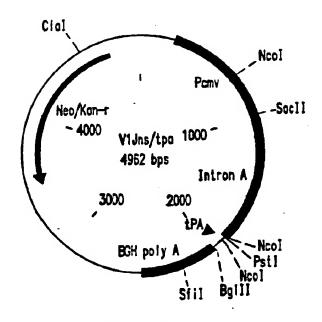


FIGURE 16

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCCTGCTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA sGinTrpProLeuThrGluGluLysIleLysAloLeuVolGluIleCysThrGluMeLGluLysGluGlyLysIleSerL 30 40 50

AGATTGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGAACGACTCCACCAAGTGGAGGAAGCTGGTG
yslieGiyProGiuAsnProTyrAsnThrProVoiPheAiolieLysLysLysAspSerThrLysTrpArgLysLeuVoi
60 '70

GACTICAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGluLeuAsnLysArgThrGinAspPheTrpGluVolGinLeuGlyIteProHisProAloGlyLeuLysLy 80 90 100

GAAGAACTCTGTGACTGTGCTGCTGCGGGGATGCCTACTTCTCTGTGCCCCTGGATGACGACTTCAGGAAGTACACTG slyslysSerVolThrVolLeu<u>Alo</u>VolGlyAspAloTyrPheSerVolProLeuAspGluAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC IoPheTnrlleProSerlleAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVoiLeuProGlnGlyTrpLysGly 140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAioliePheGinSerSerMetThrLyslieLeuGiuProPheArgLysGinAsnProAsplieVollieTyrGi 160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACCACGACCAAGATTGAGGAGCTGAGGCAGCACCC
nTyrMetAloaloLeuTyrVo1G1ySerAspLeuG1uI1eG1yG1nHisArgThrLysI1eG1uG1uLeuArgG1nHisL
190 200 210

TGCTGAGGTGGGGCCTGACCACCCTGACAAGAAGCACCAGAAGGAGCCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis 220 230

CCCGACAGTGGACTGTGCACCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG ProAspLysTrpThrVoiGinProlieVoiLeuProGiuLysAspSerTrpThrVoiAsnAspIleGinLysLeuVoiGI 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC yLysLeuAsnTrpAloSerGinlleTyrProGiylleLysVolArgGinLeuCysLysLeuLeuArgGiyThrLysAloL 270 280 290

### FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGAGCAGACAGGGAGATCCTGAAGGAGCCTGTGCAT EUThrGluVollleProLeuThrGluGluAloGluLeuGluLeuGluLeuAloGluAsnArgGluIleLeuLysGluProVolHis 300 310

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCCAGGGGCCAGTGGACCTACCAAATCTA GlyVoiTyrTyrAspProSerLysAspLeuiteAloGiuIteGinLysGinGlyGinGlyGinTrpThrTyrGinIteTy 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGInGluProPheLysAsnLeuLysThrGlyLysTyrAloArgMetArgGlyAloHisThrAsnAspVolLysGInLeuT 350 360 370

CTCAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysleuTrpTyrGinleuGluLysGluProlleVolGlyAloGluThrPheTyrVolAloGlyAloAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAioleuGinAlolleTyrLeuAloleuGinAspSerGiyLeuGiuVolAsnIieVolThrAioSerGinTyrAi
480 490 500

CCTGGGCATCATCCAGCCCGAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG ©LeuGlyllelleGlnAloGinProAspGinSerGluSerGluLeuVolAsnGinIlelleGluGinLeulleLysLysG 510 520 530

AGAACCTGTACCTGCCCTGCCCACAACCGCATTGCCGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
!ulysvoiTyrleuA!oTrpvoiProA!oHislysG!y!!eG!yG!yAsnG!uG!nVo!AsplysLeuVo!SerA!oG!y
540
550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT

1 leAr gl ysVolleuPheleuAspGIyI leAsplysAl oG InAspGIuHisGIuLysTyrHisSerAsnTrpAr gAl dMe
560 570 580

### FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTCCCCTCCTGTGACAAGTGCCAGCTGAAGCGGGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGiulleVolAloSerCysAspLysCysGlnLeuLysGlyGluA 590 600 610

GCTGTGCATGTGGCCTCCGGCTACATTGACGCTGACGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovolHisVolAloSerGlyTyrlleGluAloGluVollleProAloGluThrGlyClnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGCCTCCAACTTCACTGGGGCCACAGTGAGGGCTG uLysLeuAloGlyArgTrpProVolLysThrlleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA 670 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGlylleLysGInGluPheGlylleProTyrAsnProGInSerGInGlyVolVolAloSerMelAsn 700 710

AAGGAGCTGAAGAAGATCATTGCGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTCAT LysGluLeuLysLyslielleGlyGlnVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTICAAGAGGAAGGGGGGCATCGGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAloGlyGluArglleVolAspIleIleAloThrAspIleG
750
760
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGTuLeuGInLysGInlieThrLys1ieGInAsnPheArgVolTyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGGTGCTGCCCAG LysGtyProAtolysLeuLeuTrpLysGtyGtuGtyAtoVotVotIteGtnAspAsnSerAsplieLysVotVotProAr 800 810 820

AAAGCCCCGCCAGATC; (SEQ ID NO: 3)
Xx Boll (SEQ ID NO: 4)

FIGURE 17C

(within SEO 10 NO: 7) RoserGiulieSerAioProlieSerProlieGiuThrVoiProVoiLysLeuLysProGlyMetAspGly 20 20 CCACCOACATCTCCCCCCCATCTCCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCTGCCATGGATGCC

### FIGURE 18

WT	111 11 111 111 11 11 11 11 11 11 11 11	42
OPT	- ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC M G G K W S K R S V P G W S .	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GAT	-84
DPT	- ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC	-28
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG ETG GGA GCA	-126
OPT	- AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC	-42
WT	11 11 1 11 11 11 11 11 11 11 11 11	-168
OPT	- GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC	-56
WŢ	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- AAC ÁCC GCC GCC ÁCC ÁAC GCC GÁC TGC GCC TGG CTG GÁG GCC N T A A T N A D C A W L E A	-70
WT.	CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG Q E D E E V G F P V R P Q V	-84
WT .	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC P L R P M T Y K G A V D L S	-98
WT	- CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W O N Y T P G	-140

FIGURE 19A

WT	- CCA GGA ATC AGA TIT CCA TTG ACC TTT GGA TGG TGC TTC AAG -462	!
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG P G I R F P L T F G W C F K -154	i
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA -504	ļ
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GCC AAC GAG L V P V E P E K V E E A N E -168	3
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG -546	5
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC G E N N C L L H P M S Q H G -18	2
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC -58	3
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC  1 E D P E K E V L E W R F D -19	5
WT .	- AGC AAG CTA GCA TIT CAT CAC GTG GCC CGA GAG CTG CAT CCG -63	D
OPT	TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC S K L A F H H V A R E L H P -21	0
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30) -65	1
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQID NO:9) E Y Y K D C (SEQID NO:10) -21	6

FIGURE 19B

VIJns/nef

VIUNISTIE! PSEI CATGGGTCTTTT<u>CIGCAG</u>TCACCGTCCTTGAG<u>AICT</u>GCCACC ATG GGC GGC AAG TGG TCC AAG TCC GTG CCC . M G G K W S K R S V P

SrfJ B9JIJ

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGGAGAICIGCTGGCCTTCTAGTTGCCAGC (SEQ 1D NO: 38)

H P E Y Y K D C * (contained within SEQ 1D NO: 10:

V1Jns/nef(GZA.LLAA)

Psti Catrbosictiticiacagreaccatectigagaiciaceace atg gec ggc ang tgg tec gtg ecc M A G K W S K R S V P

SrfI BallI . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGAICTGCCAGATCAGATGCCAGC (SEQ 1D NO: 39) H P E Y Y K D C * (contained within SEQ 1D NO:14)

/lJns/tpanef & VlJns/tpanef(LLAA)

Psti Catibasictiticiocalgicaccostatatatictagatcacc atg gat gca atg ang aga ggg ctc tgc tgt gtg M D A M K R G L C C V

. . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGAGATCTGCTGGTGCCTTCTAGTTGCCAGC (SEQ ID NG: 40)

H P E Y Y K D C * (contained withon seq id no: 16.)

## FIGURE 20

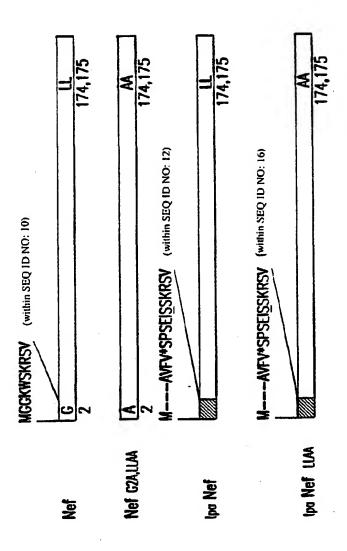


FIGURE 21

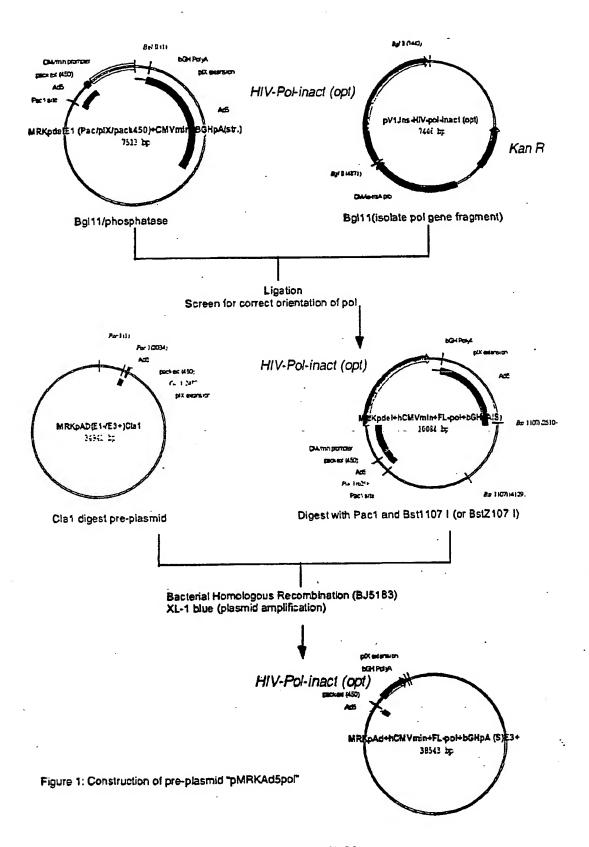


FIGURE 22

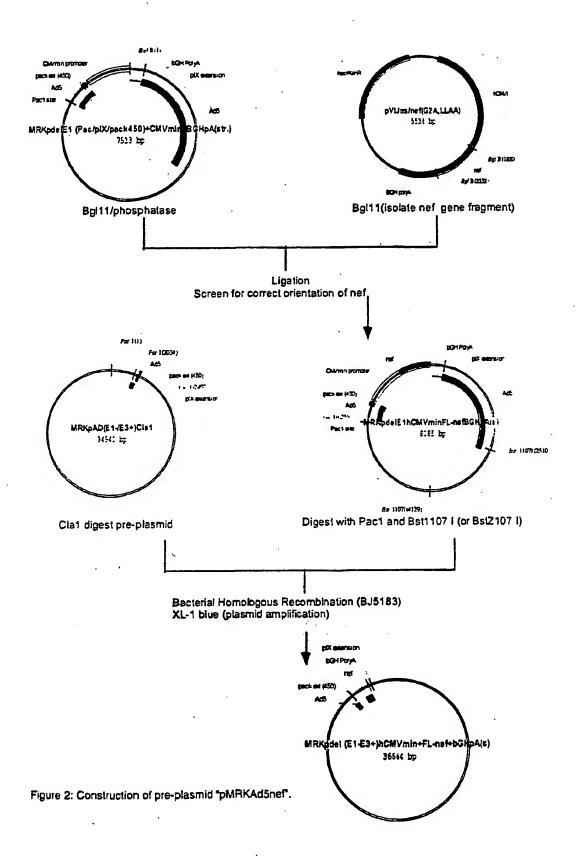
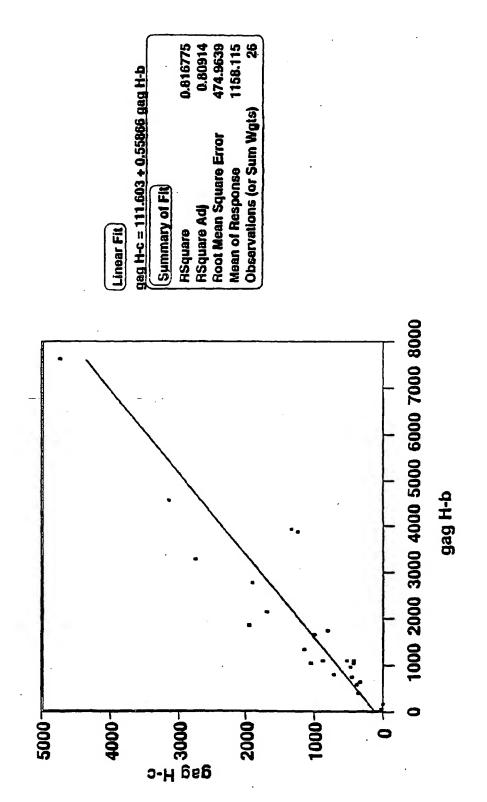
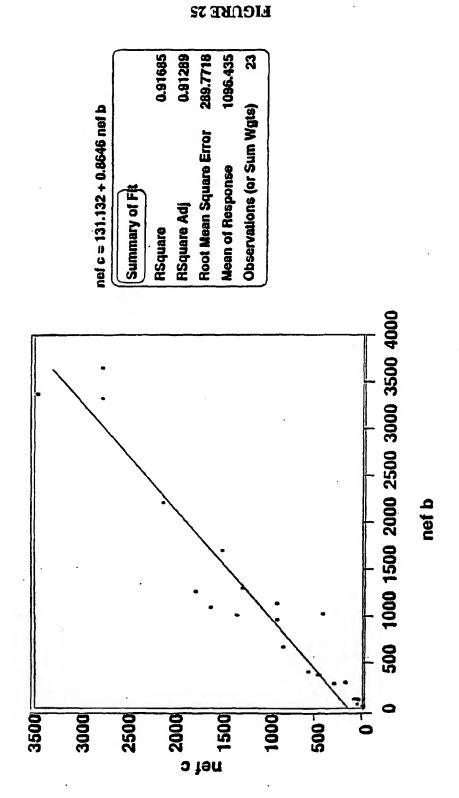


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



# Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



### MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC 51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC 101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT 151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC 201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC 251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT 301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT 351 GGGCCGCGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA 401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC 451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA 501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG 551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT 601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC 651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA 701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA 751 AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC TTTGACGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG 801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT 851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

7 i jure 26A

901	AGCGATAATG	GTACCACTAC	GCCAAAACCG	AGTACATCAA TCATGTAGTT	ACCCGCACCT
·		TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
1001		AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT
1051	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	GTAGGCGTGT CATCCGCACA	TGCCACCCTC
1101	CAGATATATT	CGTCTCGAGC	AAATCACTTG	CGTCAGATCG GCAGTCTAGC	GGACCTCTGC
1151	GGTAGGTGCG	ACAAAACTGG	AGGTATCTTC	ACACCGGGAC TGTGGCCCTG	GCTAGGTCGG
1201	AGGCGCCGGC	CCTTGCCACG	TAACCTTGCG	CGATTCCCCG CCTAAGGGGC	ACGGTTCTCA
1251	CTCTAGATGG	TACCGGGGGT	AGAGGGGGTA	TGAGACTGTG ACTCTGACAC	GGACACTTCG
1301	ACTTCGGACC	GTACCTACCG	GGGTTCCACT		GGACTGACTC
1351	CTCTTCTAGT	TCCGGGACCA	CCTTTAGACG	ACTGAGATGG TGACTCTACC	TCTTCCTCCC
1401	GTTTTAGAGG	TTCTAACCGG	GGCTCTTGGG	CTACAACACC GATGTTGTGG	GGACACAAAC
1451	GGTAGTTCTT	CTTCCTGAGG	TGGTTCACCT	GGAAGCTGGT CCTTCGACCA	CCTGAAGTCC
1501	CTCGACTTGT	TCTCCTGGGT	CCTGAAGACC	GAGGTGCAGC CTCCACGTCG	ACCCGTAGGG
1551	GGTGGGGCGA	CCGGACTTCT	TCTTCTTCAG	TGTGACTGTG ACACTGACAC	GACCGACACC
1601	CCCTACGGAT	GAAGAGACAC	GGGGACCTAC	TCCTGAAGTC	
	CGGAAGTGGT	AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	TCAGGTACCA AGTCCATGGT
	CATGTTACAC	GACGGGGTCC	CGACCTTCCC	GAGGGGACGG	ATCTTCCAGT TAGAAGGTCA
	GGAGGTACTG	GTTCTAGGAC	CTCGGGAAGT	CCTTCGTCTT	CCCTGACATT GGGACTGTAA
1801	GTGATCTACC CACTAGATGG	AGTACATGGC TCATGTACCG	TGCCCTGTAT ACGGGACATA	GTGGGCTCTG CACCCGAGAC	ACCTGGAGAT TGGACCTCTA

Figure 26B

1851	TGGGCAGCAC ACCCGTCGTG	A CCAAGA TCCTGGTTCT	TTGAGGAGCT AACTCCTCGA	GAGGCAGCAC CTCCGTCGTG	CTGCTG T GACGACTCCA
1901	GGGGCCTGAC CCCCGGACTG	CACCCCTGAC GTGGGGACTG	AAGAAGCACC TTCTTCGTGG	AGAAGGAGCC TCTTCCTCGG	CCCCTTCCTG GGGGAAGGAC
1951			CCCCGACAAG GGGGCTGTTC		
2001	CGACGGACTC	TTCCTGAGGA	GGACTGTGAA CCTGACACTT	ACTGTAGGTC	TTCGACCACC
2051		GACCCGGAGG	GTTTAGATGG	GACCGTAGTT	CCACTCCGTC
2101	GACACGTTCG	ACGACTCCCC		GACTGACTCC	ACTAGGGGGA
2151	CTGACTCCTC	CGACTCGACC	AGCTGGCTGA TCGACCGACT	CTTGTCCCTC	TAGGACTTCC
2201	TCGGACACGT	ACCCCACATG	TATGACCCCT ATACTGGGGA	GGTTCCTGGA	CTAACGACTC
2251	TAGGTCTTCG	TCCCGGTCCC	CCAGTGGACC GGTCACCTGG	ATGGTTTAGA	TGGTCCTCGG
2301	GAAGTTCTTG	GACTTCTGAC	CGTTCATACG	GTCCTACTCC	
2351	GGTTACTACA-	CTTCGTCGAC	-TGACTCCGAC	ACCICITCIA	CACCACTGAG -GTGGTGACTC
2401	AGGTAACACT	AGACCCCGTT	CTGGGGGTTC	AAGTTCGACG	
2451	CCTCTGGACC	CTCTGGACCA	CCTGACTCAT	GACCGTCCGG	ACCTGGATCC TGGACCTAGG
2501	GACTCACCCT	CAAACACTIG	TGGGGGGGG	ACCACTTCGA	GTGGTACCAG CACCATGGTC
2551	GACCTCTTCC	TCGGGTAACA	CCCCCGACTC	TGGAAGATAC	TGGCTGGGGC ACCGACCCCG
2601	ACGGTTGTCC	CTCTGGTTCG	ACCCGTTCCG	ACCGATACAC	ACCAACAGGG TGGTTGTCCC
	CGTCCGTCTT	CCACCACTGG	GACTGACTGT	CCICCIICCI	GAAGACTGCC CTTCTGACGG
	GAGGTCCGGT	AGATGGACCG	GGAGGTCCTG	AGACCGGACC	AGGTGAACAT TCCACTTGTA
2751	TGTGACTGCC ACACTGACGG	TCCCAGTATG AGGGTCATAC	CCCTGGGCAT	CATCCAGGCC GTAGGTCCGG	CAGCCTGATC

Figure 26 C

2801	AGTCTGAGTC	T CTGGTG	AACCAGATCA	TTGAGCAGCT	GATCAA G
	TCAGACTCAG	ACTCGACCAC	TTGGTCTAGT	AACTCGTCGA	CTAGTTCTTC
2851	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC	CACAAGGGCA	TTGGGGGCAA
	CTCTTCCACA	TGGACCGGAC	CCACGGACGG	GTGTTCCCGT	AACCCCCGTT
2901	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG	GTGCTGTTCC
	ACTCGTCCAC	CTGTTCGACC	ACAGACGACC	GTAGTCCTTC	CACGACAAGG
2951	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
	ACCTACCGTA	ACTGTTCCGG	GTCCTACTCG	TACTCTTCAT	GGTGAGGTTG
3001	TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCCTGTGG	TGGCTAAGGA
	ACCTCCCGAT	ACCGGAGACT	GAAGTTGGAC	GGGGGACACC	ACCGATTCCT
3051	GATTGTGGCC	TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG
	CTAACACCGG	AGGACACTGT	TCACGGTCGA	CTTCCCCCTC	CGGTACGTAC
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT	GTGGCCTCCG	GCTACATTGA
	CTCCCGTTCC	ACTAGGACCA	CCGACACGTA	CACCGGAGGC	CGATGTAACT
3201	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC	TACTTCCTGC
	CCGACTCCAC	TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG	ATGAAGGACG
3251	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301	TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT
	AGGTTGAAGT	GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351	CAAGCAGGAG GTTCGTCCTC	TTTGGCATCC AAACCGTAGG	CCTACAACCC GGATGTTGGG	CCAGTCCCAG GGTCAGGGTC	GGGGTGGTGG
3401	CCTCCATGAA	CAAGGAGCTG	AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG
	GGAGGTACTT	GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451		TGAAGACAGC ACTTCTGTCG			
3501	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG	AGGATTGTGG
	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	GCGACCCCTC	TCCTAACACC
3551	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
	TGTAGTAACG	GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
3601	AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG
	TTCTAGGTCT	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
3651	GAAGGGCCCT	GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC
	CTTCCCGGGA	CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	CACCACTAGG
3701	AGGACAACTC	TGACATCAAG	GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC
	TCCTGTTGAG	ACTGTAGTTC	CACCACGGGT	CCTCCTTCCG	GTTCTAGTAG

Figure 26 D

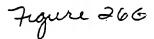
3751	AGGGACTATG	AGCAGAT	GGCTGGGGAT	GACTGTGTGGT	CCTCCA TA
	TCCCTGATAC	COTTCGTCTA	CCGACCCCTA	CTGACACACC	GGAGGT GT
3801	GGATGAGGAC	TAAAGCCCGG	GCAGATCTGC	TGTGCCTTCT	AGTTGCCAGC
	CCTACTCCTG	ATTTCGGGCC	CGTCTAGACG	ACACGGAAGA	TCAACGGTCG
3851	CATCTGTTGT	TTGCCCCTCC	CCCGTGCCTT	CCTTGACCCT	GGAAGGTGCC
	GTAGACAACA	AACGGGGAGG	GGGCACGGAA	GGAACTGGGA	CCTTCCACGG
3901	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	CGCATTGTCT
	TGAGGGTGAC	AGGAAAGGAT	TATTTTACTC	CTTTAACGTA	GCGTAACAGA
3951	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG
	CTCATCCACA	GTAAGATAAG	ACCCCCACC	CCACCCGTC	CTGTCGTTCC
4001	GGGAGGATTG CCCTCCTAAC	GGAAGACAAT CCTTCTGTTA	AGCAGGCATG TCGTCCGTAC	CTGGGGATGC GACCCCTACG	CCACCCGAGA
4051	ATGGCCGATC TACCGGCTAG	CCGCGCGCCA	ACTGAAATGT TGACTTTACA	GTGGGCGTGG CACCCGCACC	CTTAAGGGTG GAATTCCCAC
4101	GGAAAGAATA	TATAAGGTGG	GGGTCTTATG	TAGTTTTGTA	TCTGTTTTGC
	CCTTTCTTAT	ATATTCCACC	CCCAGAATAC	ATCAAAACAT	AGACAAAACG
4151	AGCAGCCGCC	GCCGCCATGA	GCACCAACTC	GTTTGATGGA	AGCATTGTGA
	TCGTCGGCGG	CGGCGGTACT	CGTGGTTGAG	CAAACTACCT	TCGTAACACT
4201	GCTCATATTT CGAGTATAAA	GACAACGCGC CTGTTGCGCG	ATGCCCCCAT TACGGGGGTA	GGGCCGGGT	GCGTCAGAAT CGCAGTCTTA
4251	GTGATGGGCT CACTACCCGA	CCAGCATTGA GGTCGTAACT	TGGTCGCCCC	GTCCTGCCCG CAGGACGGGC	CAAACTCTAC GTTTGAGATG
4301	TACCTTGACC	TACGAGACCG	TGTCTGGAAC	GCCGTTGGAG	ACTGCAGCCT
	ATGGAACTGG	ATGCTCTGGC	ACAGACCTTG	CGGCAACCTC	TGACGTCGGA
4351	666666666 6666666666666666666666666666	TTCAGCCGCT AAGTCGGCGA	GCAGCCACCG CGTCGGTGGC	CCCGCGGGAT	TGTGACTGAC ACACTGACTG
4401	TTTGCTTTCC	TGAGCCCGCT	TGCAAACAGT	GCAGCTTCCC	GTTCATCCGC
	AAACGAAAGG	ACTCGGGCGA	ACGTTTGTCA	CGTCGAAGGG	CAAGTAGGCG
4451	GGCGCTACTG	TTCAACTGCC	GAGAAAACCG	TGTTAACCTA	TCTTTGACCC AGAAACTGGG
	CCCTTGAATT	ACAGCAAAGA	. GTCGTCGACA	ACCTAGACGC	CCAGCAGGTT
4551	TCTGCCCTGA	AGGCTTCCTC	CCCTCCCAAT	GCGGTTTAAA	ACATAAATAA
	AGACGGGACT	TCCGAAGGAG	GGGAGGGTTA	CGCCAAATTT	TGTATTTATT
4501	AAAACCAGAC	TCTGTTTGGA	TTTGGATCAP	GCAAGTGTCT	TGCTGTCTTT
	TTTTGGTCTG	AGACAAACCT	AAACCTAGTT	CGTTCACAGA	ACGACAGAAA
4651	ATTTAGGGGT TAAATCCCCA	TTTGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	CGGTAGGCCC CGCCATCCGGC	GGGACCAGCG CCCTGGTCGC	CAGAGCCAGC

Figure 26E

4701	TTGAGGGTCC AACTCCCAGG	TCTGTATTTT ATAAAA	TTCCAGGACG AAGGTCCTGC	TGGTAAAGGT ACCATTTCCA	-GACTCPCGAT CTGAGA A
4751				GGGGTGGAGG CCCCACCTCC	
4801	GCAGAGCTTC CGTCTCGAAG	ATGCTGCGGG TACGACGCCC	GTGGTGTTGT CACCACAACA	AGATGATCCA TCTACTAGGT	GTCGTAGCAG CAGCATCGTC
4851				TTCAGTAGCA AAGTCATCGT	
4901	CAGGGGCAGG GTCCCCGTCC	CCCTTGGTGT GGGAACCACA	AAGTGTTTAC TTCACAAATG	AAAGCGGTTA TTTCGCCAAT	AGCTGGGATG TCGACCCTAC
4951	CCACGTATGC	ACCCCTATAC	TCTACGTAGA	TGGACTGTAT ACCTGACATA	AAAATCCAAC
5001	CGATACAAGG	GTCGGTATAG	GGAGGCCCCT	TTCATGTTGT AAGTACAACA	CGTCTTGGTG
5051	GTCGTGTCAC	ATAGGCCACG	TGAACCCTTT	TTTGTCATGT AAACAGTACA	TCGAATCTTC
5101	CTTTACGCAC	CTTCTTGAAC	CTCTGCGGGA	TGTGACCTCC ACACTGGAGG	TTCTAAAAGG
5151				CCACGGGCGG GGTGCCCGCC	
5201	CTTCTATAAA	GACCCTAGTG	ATTGCAGTAT	GTTGTGTTCC CAACACAAGG	TCCTACTCTA
5251	GCAGTATCCG	GTAAAAATGT	TTCGCGCCCG	GGAGGGTGCC CCTCCCACGG	TCTGACGCCA
5301	TATTACCAAG	GTAGGCCGGG	TCCCCGCATC	TTACCCTCAC AATGGGAGTG	TCTAAACGTA
5351	AAGGGTGCGA	AACTCAAGTC	TACCCCCTA	CATGTCTACC GTACAGATGG	ACGCCCCGCT
5401	TGAAGAAAAC ACTTCTTTTG	GGTTTCCGGG CCAAAGGCCC	GTAGGGGAGA CATCCCCTCT	TCAGCTGGGA AGTCGACCCT	AGAAAGCAGG TCTTTCGTCC
5451	AAGGACTCGT	CGACGCTGAA	TGGCGTCGGC	GTGGGCCCGT CACCCGGGCA	TTTAGTGTGG
		ACGTTGACCA	TCAATTCTCT	CGACGTCGAC	GGCAGTAGGG
		CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	CTGACCAAAT GACTGGTTTA	CCGCCAGAAG GGCGGTCTTC	CCCCTCCCCC	CCCAGCGATA GGGTCGCTAT	GCAGTTCTTG CGTCAAGAAC

Figure 26F

5651	CAAGGAAGCA GTTCCTTCGT			ACCGTCCGCC TGGCAGGCGG	
5701				GGTCCCACAG CCAGGGTGTC	
5751				CCTCGTTTCG GGAGCAAAGC	
5801				TCGTCCAGAC AGCAGGTCTG	
5851				CAGCGTAGTC GTCGCATCAG	
5901				CCAGGGTGCG GGTCCCACGC	
5951	CAGGACGACC	ACGACTTCGC	GACGGCCAGA	TCGCCCTGCG AGCGGGACGC	GCAGCCGGTC
6001	GTAGCATTTG CATCGTAAAC	ACCATGGTGT TGGTACCACA	CATAGTCCAG GTATCAGGTC	CCCCTCCGCG	GCGTGGCCCT
6051				CGCACGAGGG GCGTGCTCCC	
6101				AATACCGATT TTATGGCTAA	
6151				CTCGCATTCC GAGCGTAAGG	
6201				GGTTTCCCCC	
6251				CGGTGTCCAC GCCACAGGTG	
6301				CTTGAGAGGC GAACTCTCCG	
6351					CTCTGAGACA GAGACTCTGT
6401	AAGGCTCGCG TTCCGAGCGC	TCCAGGCCAG AGGTCCGGTC	CACGAAGGAG GTGCTTCCTC	GCTAAGTGGG CGATTCACCC	AGGGGTAGCG TCCCCATCGC
6451	GTCGTTGTCC CAGCAACAGG	ACTAGGGGGT TGATCCCCCA	CCACTCGCTC GGTGAGCGAG	CAGGGTGTGA GTCCCACACT	AGACACATGT TCTGTGTACA
6501	CGCCCTCTTC GCGGGAGAAG	GGCATCAAGG CCGTAGTTCC	AAGGTGATTG TTCCACTAAC	GTTTGTAGGT CAAACATCCA	GTAGGCCACG CATCCGGTGC
					GGGCGCGTTC CCCGCGCAAG



6601	GTCCTCACTC CAGGAGTGAG	TCTTCCGCAT AGGCGTA	CGCTGTCTGC GCGACAGACG	CACCCCCACC. CTCCCCGCTCG	ACAACO C
6651	AGTACTCCCT TCATGAGGGA	CTGAAAAGCG GACTTTTCGC	GGCATGACTT CCGTACTGAA	CTGCGCTAAG GACGCGATTC	ATTGTCAGTT TAACAGTCAA
6701	TCCAAAAACG AGGTTTTTGC	AGGAGGATTT TCCTCCTAAA	GATATTCACC CTATAAGTGG	TGGCCCGCGG ACCGGGCGCC	TGATGCCTTT ACTACGGAAA
6751	GAGGGTGGCC CTCCCACCGG	GCATCCATCT CGTAGGTAGA	GGTCAGAAAA CCAGTCTTTT	GACAATCTTT CTGTTAGAAA	TTGTTGTCAA AACAACAGTT
6801	GCTTGGTGGC CGAACCACCG	AAACGACCCG TTTGCTGGGC	TAGAGGGCGT ATCTCCCGCA	TGGACAGCAA ACCTGTCGTT	CTTGGCGATG GAACCGCTAC
6851	GAGCGCAGGG CTCGCGTCCC	TTTGGTTTTT AAACCAAAAA	GTCGCGATCG CAGCGCTAGC	GCGCGCTCCT CCCGCGAGGA	TGGCCGCGAT ACCGGCGCTA
6901	CAAATCGACG	TGCATAAGCG	CGCGTTGCGT	CCGCCATTCG GGCGGTAAGC	CCTTTCTGCC
6951	ACCACGCGAG	CAGCCCGTGG	TCCACGTGCG	GCCAACCGCG CGGTTGGCGC	CAACACGTCC
7001	CACTGTTCCA	GTTGCGACCA	CCGATGGAGA	CCGCGTAGGC GGCGCATCCG	CGAGCAACCA
7051	GGTCGTCTCC	GCCGGCGGGA	ACGCGCTCGT	GAATGGCGGT CTTACCGCCA	TCCCCCAGAT
7101	CGACGCAGAG	CAGGCCCCCC	AGACGCAGGT	CGGTAAAGAC GCCATTTCTG	GGGCCCGTCG
7151	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	CCTTGCAAGT GGAACGTTCA	GATCGCGGAC
7201	GACGGTACGC	GCCCGCCGTT	CGCGCGCGAG	GTATGGGTTG CATACCCAAC	TCACCCCCTG
7251	GGGTACCGTA	CCCCACCCAC	TCGCGCCTCC	CGTACATGCC GCATGTACGG	CGTTTACAGC
7301	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	AGATATGTAG TCTATACATC	CCATCGTAGA
		TACGACCGCG	CGTGCATTAG	CATATCAAGC	ACGCTCCCTC
		CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	
7501	GAAGACGTTG CTTCTGCAAC	AAGCTGGCGT TTCGACCGCA	CTGTGAGACC GACACTCTGG	TACCGCGTCA ATGGCGCAGT	CGCACGAAGG GCGTGCTTCC

Figure 26 H

7551	AGGCGTAGGA TCCGCATCCT	G CGCAGC CAGCGCGTCG	TTGTTGACCA AACAACTGGT	GCTCGGCGGT CGAGCCGCCA	CTCGACCTCC
7601	TCTAGGGCGC AGATCCCGCG	'AGTAGTCCAG TCATCAGGTC	GGTTTCCTTG CCAAAGGAAC	ATGATGTCAT TACTACAGTA	ACTTATCCTG TGAATAGGAC
7651	TCCCTTTTTT AGGGAAAAAA	TTCCACAGCT AAGGTGTCGA	CGCGGTTGAG GCGCCAACTC	GACAAACTCT CTGTTTGAGA	TCGCGGTCTT AGCGCCAGAA
7701	TCCAGTACTC AGGTCATGAG	TTGGATCGGA AACCTAGCCT	AACCCGTCGG TTGGGCAGCC	CCTCCGAACG GGAGGCTTGC	GTAAGAGCCT CATTCTCGGA
7751	TCGTACATCT	ACTGGTTGAC TGACCAACTG	CCGGACCATC	CGCGTCGTAG	GGAAAAGATG
7801	CCCATCGCGC	TATGCCTGCG ATACGGACGC	GCCGGAAGGC	CTCGCTCCAC	ACCCACTCGC
7851	GTTTCCACAG	CCTGACCATG GGACTGGTAC	TGAAACTCCA	TGACCATAAA	CTTCAGTCAC
7901	AGCAGCGTAG	CGCCCTGCTC GCGGGACGAG	CCTCTCCTTT	TTCAGGCACG	CGAAAAACCT
7951	TGCGCCTAAA	GGCAGGGCGA CCGTCCCGCT	TCCACTGTAG	CAACTTCTCA	TAGAAAGGGC
8001	GCGCTCCGTA	AAAGTTGCGT TTTCAACGCA	CACTACGCCT	TCCCAGGGCC	GTGGAGCCTT
8051	GCCAACAATT		CCGCTCGTGC	TAGAGCAGTT	TCGGCAACTA
8101	CAACACCGGG	ACAATGTAAA TGTTACATTT	CAAGGTTCTT	CGCGCCCTAC	GGGAACTACC
8151	TTCCGTTAAA	AAATTCAAGG	AGCATCCACT	CGAGAAGTCC	GGAGCTGAGC
8201	GGCACGAGAC	TTTCCCGGGT	CAGACGTTCT	ACTCCCAACC	AAGCGACGAA
8251	ACTCGAGGTG	TCCAGTGCCC	GGTAATCGTA	AACGTCCACC	TCGCGAAAGG AGCGCTTTCC
	AGGATTTGAC	CGCTGGATAC	CGGTAAAAA	GACCCCACTA	GCAGTAGAAG CGTCATCTTC
	CATTCGCCCA	GAACAAGGGT	CGCCAGGGTA	GGTTCCAAGC	CGGCTAGGTC CGCCGATCCAG
8401	AGCGCGCCGT	CAGTGATCTC	CGACTAGAGG	CGGCTTGAAC	ATGACCAGCA TACTGGTCGT
8451	TGAAGGGCAC ACTTCCCGTG	GAGCTGCTTC CTCGACGAAG	CCAAAGGCCC GGTTTCCGGG	CCATCCAAG GGTAGGTTCA	TATCCAGAGA

Figure 26I

8501	ACATCGTAGG TGTAGCATCC	TAAAGAG ACTGTTTCTC	ACGCTCGGTG TGCGAGCCAC	CGAGGATGCG GCTCCTACGC	AGCCGA GG TCGGCTAGCC
8551	GAAGAACTGG CTTCTTGACC	ATCTCCCGCC TAGAGGGCGG	ACCAATTGGA TGGTTAACCT	GGAGTGGCTA CCTCACCGAT	TTGATGTGGT AACTACACCA
8601	CTTTCATCTT	CAGGGACGCT	GCCCGCCTTG	ACTCGTGCTG TGAGCACGAC	CGAAAACATT
8651	TTTGCACGCG	TCATGACCGT	CGCCACGTGC	GGCTGTACAT CCGACATGTA	GGACGTGCTC
8701	CAACTGGACT	GCTGGCGCGT	GTTCCTTCGT	GAGTGGGAAT CTCACCCTTA	AACTCGGGGA
8751	GCGGACCGCC	CAAACCGACC	ACCAGAAGAT	CTTCGGCTGC GAAGCCGACG	AACAGGAACT
8801	GGCAGACCGA	CGAGCTCCCC	TCAATGCCAC	GATCGGACCA CTAGCCTGGT	GGTGCGGCGC
8851	GCTCGGGTTT	CAGGTCTACA	GGCGCGCGCC	CGGTCGGAGC GCCAGCCTCG	AACTACTGTT
8901	GTAGCGCGTC	TACCCTCGAC	AGGTACCAGA	GGAGCTCCCG CCTCGAGGGC	GCCGCAGTCC
8951	TCAGGCGGGA AGTCCGCCCT	GCTCCTGCAG CGAGGACGTC	GTTTACCTCG CAAATGGAGC	CATAGACGGG GTATCTGCCC	TCAGGGCGCG AGTCCCGCGC
9001	CCGATCTAGG	TCCACTATGG	ATTAAAGGTC	GGGCTGGTTG CCCGACCAAC	CACCGCCGCA
9051	GCTACCGAAC	GTTCTCCGGC	GTAGGGGCGC	GCGCGACTAC CGCGCTGATG	CCATGGCGCG
9101	CCGCCCGCCA	CCCGGCGCCC	CCACAGGAAC	GATGATGCAT CTACTACGTA	GATTTTCGCC
9151	ACTGCGCCCG	CTCGGGGGCC	TCCATCCCCC	GGCTCCGGAC CCGAGGCCTG	GGCGGCCCTC
9201	TCCCCCGTCC	CCGTGCAGCC	GCGGCGCGCG	GGGCAGGAGC	ACCACGACGC
		CGACCGCTTG	CGCTGCTGCG	CCGCCAACTA	GAGGACTTAG
9301	TGGCGCCTCT ACCGCGGAGA	GCGTGAAGAC CGCACTTCTG	GACGGGCCCG CTGCCCGGCC	GTGAGCTTGA CACTCGAACT	ACCTGAAAGA TGGACTTTCT
9351	GAGTTCGACA CTCAAGCTGT	GAATCAATTT CTTAGTTAAA	CGGTGTCGTT GCCACAGCAA	CACGCCGCCG	TGGCGCAAAA ACCGCGTTTT
9401	TCTCCTGCAC AGAGGACGTG	CAGAGGACTC	TTGTCTTGAT AACAGAACTA	AGGCGATCTC TCCGCTAGAG	GGCCATGAAC CCGGTACTTG

Figure 26 J

9451	TGCTCGATCT ACGAGCTAGA	CTCCTG GAAGGAGGAC	GAGATCTCCG CTCTAGAGGC	CGTCCGGCTC GCAGGCCGAG	CCTCCA T CGAGGTGCCA
9501	GCCGCCGAGG			GAGCTGCGAG CTCGACGCTC	
9551	GGCCTCCCTC CCGGAGGGAG			CCACGCCCCC GGTGCGGGGG	
9601				AGCTCCACGT TCGAGGTGCA	
9651			-	GTAGTTGAGG CATCAACTCC	
9701				AGCGTCGCAA TCGCAGCGTT	
9751		GGTTCCGGAG	TTCCGCGAGG	TACCGGAGCA	TCTTCAGGTG
9801	CCGCTTCAAC	TTTTTGACCC	TCAACGCGCG	CGACACGGTT GCTGTGCCAA	TTGAGGAGGA
9851	GGTCTTCTGC	CTACTCGAGC	CGCTGTCACA	CGCGCACCTC	CGCGAGTTTC
9901				TCCTCTTCCA AGGAGAAGGT	
9951				AGGGGGGACA TCCCCCTGT	CGGCGGCGAC GCCGCCGCTG -
10001	CTGCCGCGTG	GCCCTCCGCC	AGCTGTTTCG	GCTCGATCAT CGAGCTAGTA	GAGGGGCGCC
10051	GCTGCCGCGT	ACCAGAGCCA	CTGCCGCGCC	CCGTTCTCGC GGCAAGAGCG	CCCCCCCCTC
10101	AACCTTCTGC	GGCGGGCAGT	ACAGGGCCAA	TACCCAACCG	•
10151	GTACGCCGTC	CCTATGCCGC	GATTGCTACG	TAGAGTTGTT	
10201	GGTACTCCGC CCATGAGGCG	CGCCGAGGGA GCGGCTCCCT	CCTGAGCGAG GGACTCGCTC	TCCGCATCGA AGGCGTAGCT	CCGGATCGGA GGCCTAGCCT
	TTTGGAGAGC	TCTTTCCGCA	GATTGGTCAG	TGTCAGCGTT	GGTAGGCTGA CCATCCGACT
	CGTGGCACCG	CCCGCCGTCG	CCCGCCGCCA	GCCCCAACAA	TCTGGCGGAG AGACCGCCTC
10351	GTGCTGCTGA CACGACGACT	TGATGTAATT ACTACATTAA	AAAGTAGGCG TTTCATCCGC	GTCTTGAGAC CAGAACTCTG	GGCGGATGGT CCGCCTACCA

Figure 26 K

10401	CGACAGAAGC	A TGTCCT	TGGGTCCGGC	CTGCTGAATG	CCCACCOTA
	GCTGTCTTCG	TACAGGA	ACCCAGGCCG	GACGACTTAC	CCCTCCCA
10451	CGGCCATGCC	CCAGGCTTCG	TTTTGACATC	GGCGCAGGTC	TTTGTAGTAG
	GCCGGTACGG	GGTCCGAAGC	AAAACTGTAG	CCGCGTCCAG	AAACATCATC
10501	TCTTGCATGA	GCCTTTCTAC	CGGCACTTCT	TCTTCTCCTT	CCTCTTGTCC
	AGAACGTACT	CGGAAAGATG	GCCGTGAAGA	AGAAGAGGAA	GGAGAACAGG
10551				GGCGGAGTTT CCGCCTCAAA	
10601	GGCGCCCTCT	TCCTCCCATG	CGTGTGACCC	CGAAGCCCCT	CATCGGCTGA
	CCGCGGGAGA	AGGAGGGTAC	GCACACTGGG	GCTTCGGGGA	GTAGCCGACT
10651	AGCAGGGCTA	GCTCGGCGAC	AACGCGCTCG	GCTAATATGG	CCTGCTGCAC
	TCGTCCCGAT	CCAGCCGCTG	TTGCGCGAGC	CGATTATACC	GGACGACGTG
10701				GTCCACAAAG CAGGTGTTTC	
10751	CGCCCGTGTT	GATGGTGTAA	GTGCAGTTGG	CCATAACGGA	CCAGTTAACG
	GCGGGCACAA	CTACCACATT	CACGTCAACC	GGTATTGCCT	GGTCAATTGC
10801	GTCTGGTGAC	CCGGCTGCGA	GAGCTCGGTG	TACCTGAGAC	GCGAGTAAGC
	CAGACCACTG	GGCCGACGCT	CTCGAGCCAC	ATGGACTCTG	CGCTCATTCG
10851	CCTCGAGTCA	AATACGTAGT	CGTTGCAAGT	CCGCACCAGG	TACTGGTATC
	GGAGCTCAGT	TTATGCATCA	GCAACGTTCA	GGCGTGGTCC	ATGACCATAG
10901	CCACCAAAAA	GTGCGGCGGC	GGCTGGCGGT	AGAGGGGCCA	GCGTAGGGTG
	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	TCTCCCCGGT	CGCATCCCAC
10951	GCCGGGGCTC	CGGGGGCGAG	ATCTTCCAAC	ATAAGGCGAT	GATATCCGTA
	CGGCCCCGAG	GCCCCGCTC	TAGAAGGTTG	TATTCCGCTA	CTATAGGCAT
11001	GATGTACCTG CTACATGGAC	GACATCCAGG CTGTAGGTCC	TGATGCCGGC ACTACGGCCG	GGCGGTGGTG CCGCCACCAC	GAGGCGCGCGC
11051	GAAAGTCGCG	GACGCGGTTC	CAGATGTTGC	GCAGCGGCAA	AAAGTGCTCC
	CTTTCAGCGC	CTGCGCCAAG	GTCTACAACG	CGTCGCCGTT	TTTCACGAGG
11101	ATGGTCGGGA	CGCTCTGGCC	GGTCAGGCGC	GCGCAATCGT	TGACGCTCTA
	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	CGCGTTAGCA	ACTGCGAGAT
11151	GACCGTGCAA	AAGGAGAGCC	TGTAAGCGGG	CACTCTTCCG	TGGTCTGGTG
	CTGGCACGTT	TTCCTCTCGG	ACATTCGCCC	GTGAGAAGGC	ACCAGACCAC
11201	CATAAATTCG	CAAGGGTATC	ATGGCGGACG	ACCGGGGTTC	GAGCCCCGTA
	CTATTTAAGC	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	CTCGGGGCAT
11251	TCCGGCCGTC AGGCCGGCAG	CGCCGTGATC GCGGCACTAG	CATGCGGTTA GTACGCCAAT	CCGCCGCGT	GTCGAACCCA CAGCTTGGGT
11301	GGTGTGCGAC	GTCAGACAAC	GGGGGAGTGC	TCCTTTTGGC	TTCCTTCCAG
	CCACACGCTG	CAGTCTGTTG	CCCCTCACG	AGGAAAACCG	AAGGAAGGTC

Figure 26L

11351	CCCCCCCCCC	T TGCGCTA ACACGCGAT	GCTTTTTTGG CGAAAAAACC	CCACTGGCCG GGTGACCGGC	CGCGCA TT GCGCGT CCA
11401	AAGCGGTTAG TTCGCCAATC			AGTGGCTCGC TCACCGAGCG	
11451				GGGACCCCG CCCTGGGGGC	
11501				TTGCCTCCCC AACGGAGGGG	
11551				GACGAGCCCC CTGCTCGGGG	
11601				CGCGGGGGGA	
11651				GGGCACCCTC CCCGTGGGAG	
11701				GACGCGGCAG CTGCGCCGTC	CAGATGGTGA GTCTACCACT
11751				CTACCTGGAC GATGGACCTG	
11801				CTCCTGAGCG GAGGACTCGC	
11851				TACGTGCCGC ATGCACGGCG	
11901				GGAGATGCGG CCTCTACGCC	
11951				TGAATCGCGA ACTTAGCGCT	
12001				ACCGGGATTA TGGCCCTAAT	
12051				CGCATACGAG GCGTATGCTC	
12101	ACCAGGAGAT TGGTCCTCTA				GCGTACGCTT CGCATGCGAA
12151	GTGGCGCGCGC CACCGCGCGC				
12201	AAGCGCGCTG TTCGCGCGAC				
12251	TCCTTATAGT AGGAATATCA				

7 igure 26 M

12301	CTAAACATAG GATTTGTATC	ATCTCGGGCT	CCCGGCGACC	GACGAGCTAA	ACTATTTTA
12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG	GCTGACAAGG
	GGACGTCTCG	TATCACCACG	TCCTCGCGTC	GAACTCGGAC	CGACTGTTCC
12401	TGGCCGCCAT	CAACTATTCC	ATGCTTAGCC	TGGGCAAGTT	TTACGCCCGC
	ACCGGCGGTA	GTTGATAAGG	TACGAATCGG	ACCCGTTCAA	AATGCGGGCG
12451	AAGATATACC	ATACCCCTTA	CGTTCCCATA	GACAAGGAGG	TAAAGATCGA
	TTCTATATGG	TATGGGGAAT	GCAAGGGTAT	CTGTTCCTCC	ATTTCTAGCT
12501	GGGGTTCTAC	ATGCGCATGG	CGCTGAAGGT	GCTTACCTTG	AGCGACGACC
	CCCCAAGATG	TACGCGTACC	GCGACTTCCA	CGAATGGAAC	TCGCTGCTGG
12551	TGGGCGTTTA	TCGCAACGAG	CGCATCCACA	AGGCCGTGAG	CGTGAGCCGG
	ACCCGCAAAT	AGCGTTGCTC	GCGTAGGTGT	TCCGGCACTC	GCACTCGGCC
12601	CGCGCGAGC	TCAGCGACCG	CGAGCTGATG	CACAGCCTGC	AAAGGGCCCT
	GCCGCGCTCG	AGTCGCTGGC	GCTCGACTAC	GTGTCGGACG	TTTCCCGGGA
12651	GGCTGGCACG	GGCAGCGGCG	ATAGAGAGGC	CGAGTCCTAC	TTTGACGCGG
	CCGACCGTGC	CCGTCGCCGC	TATCTCTCCG	GCTCAGGATG	AAACTGCGCC
12701	GCGCTGACCT	GCGCTGGGCC	CCAAGCCGAC	GCGCCCTGGA	GCCAGCTGGG
	CGCGACTGGA	CGCGACCCGG	GGTTCGGCTG	CGCGGGACCT	CCGTCGACCC
12751	GCCGGACCTG	GGCTGGCGGT	GGCACCCGCG	CGCGCTGGCA	ACGTCGGCGG
	CGGCCTGGAC	CCGACCGCCA	CCGTGGGCGC	GCGCGACCGT	TGCAGCCGCC
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT
	GCACCTCCTT	ATACTGCTCC	TGCTACTCAT	GCTCGGTCTC	CTGCCGCTCA
12851	ACTAAGCGGT	GATGTTTCTG	ATCAGATGAT	GCAAGACGCA	ACGGACCCGG
	TGATTCGCCA	CTACAAAGAC	TAGTCTACTA	CGTTCTGCGT	TGCCTGGGCC
12901	CGGTGCGGGC	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC
	GCCACGCCCG	CCGCGACGTC	TCGGTCGGCA	GGCCGGAATT	GAGGTGCCTG
12951	GACTGGCGCC	AGGTCATGGA	CCGCATCATG	TCGCTGACTG	CGCGCAATCC
	CTGACCGCGG	TCCAGTACCT	GGCGTAGTAC	AGCGACTGAC	GCGCGTTAGG
13001	TGACGCGTTC	CGGCAGCAGC	CGCAGGCCAA	CCGGCTCTCC	GCAATTCTGG
	ACTGCGCAAG	GCCGTCGTCG	GCGTCCGGTT	GGCCGAGAGG	CGTTAAGACC
13051	AAGCGGTGGT TTCGCCACCA	CCCGCCGCGC	GCAAACCCCA CGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GGTGCTGGCG CCACGACCGC
13101	ATCGTAAACG	CGCTGGCCGA	AAACAGGGCC	ATCCGGCCCG	ACGAGGCCGG
	TAGCATTTGC	GCGACCGGCT	TTTGTCCCGG	TAGGCCGGGC	TGCTCCGGCC
13151	CCTGGTCTAC	GACGCGCTGC	TTCAGCGCGT	GGCTCGTTAC	AACAGCGGCA
	GGACCAGATG	CTGCGCGACG	AAGTCGCGCA	CCGAGCAATG	TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG
	TGCACGTCTG	GTTGGACCTG	GCCGACCACC	CCCTACACGC	GCTCCGGCAC

Figure 26 N.

13251		GCAGCAGGGC CGTCGTCCCG	
13301		CACAGCCCGC GTGTCGGGCG	
13351		AGCGCACTGC TCGCGTGACG	
13401		GTCTGGGCCA CAGACCCGGT	
13451	 	TAAACCTGAG ATTTGGACTC	
13501		GCTCCCACAG CGAGGGTGTC	
13551		GCGCCTGTTG CGCGGACAAC	
13601	 	CCCGGGACAC GGGCCCTGTG	
13651		GGTCAGGCGC CCAGTCCGCG	
13701	 	CCGCGCGCTG	
13751	 	ACCTGCTGAC TGGACGACTG	
13801		AGCGAGGAGG TCGCTCCTCC	
13851		CCTGATGCGC GGACTACGCG	
13901	 	GCAACATGGA CGTTGTACCT	
13951	 	CTAATGGACT GATTACCTGA	
14001			ACTGGCTACC TGACCGATGG
14051			GGTAACGATG CCATTGCTAC
14101			GCAACCGCAG CGTTGGCGTC
14151			CGCTGCGAAA GCGACGCTTT

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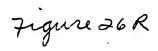
14201	CCTTTCGAAG	CCCGGTT	GCAGCTTGTC CGTCGAACAG	GCTAGATCCG	CGACGC G
14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATÇACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	CCCCCCCCCC	CCACCCGTCG GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	TTTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACŢCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT	TAGTATGCGG	CGCGCGCGA	TGTATGAGGA	AGGTCCTCCT
	CATAAGGGGA	ATCATACGCC	GCGCGCCGCT	ACATACTCCT	TCCAGGAGGA
14751	CCCTCCTACG	AGAGTGTGGT	GAGCGCGGCG	CCAGTGGCGG	CGGCGCTGGG
	GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
14801	TTCTCCCTTC AAGAGGGAAG	GATGCTCCCC CTACGAGGGG	TGGACCCGCC ACCTGGGCGG	GTTTGTGCCT CAAACACGGA	CCGCGGTACC
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA AAGTTTTGTT	TGACTACAGC ACTGATGTCG	CCGGGGGAGG	CAAGCACACA GTTCGTGTGT	GACCATCAAT CTGGTAGTTA
15051	CTTGACGACC	GGTCGCACTG	GGGCGGCGAC	CTGAAAACCA	TCCTGCATAC
	GAACTGCTGG	CCAGCGTGAC	CCCGCCGCTG	GACTTTTGGT	AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

15151	GGGTGATGGT	CECTTG	CCTACTAAGG	ACAATCAGGT	GGAGCT
	CCCACTACCA	CAGCGCGAAC	GGATGATTCC	TGTTAGTCCA	CCTCGACTTT
15201		TGGAGTTCAC			
	ATGUTCACCC	ACCTCAAGTG	CGACGGGCTC	CCGTTGATGA	GGCTCTGGTA
15251		CTTATGAACA			
	CTGGTATCTG	GAATACTTGT	TGCGCTAGCA	CCTCGTGATG	AACTTTCACC
15301		CGGGGTTCTG			
	CGTCTGTCTT	GCCCCAAGAC	CTTTCGCTGT	AGCCCCATTT	CAAACTGTGG
15351		GACTGGGGTT			
	GCGTTGAAGT	CTGACCCCAA	ACTGGGGCAG	TGACCAGAAC	AGTACGGACC
15401	GGTATATACA	AACGAAGCCT	TCCATCCAGA	CATCATTTG	CTGCCAGGAT
	CCATATATGT	TTGCTTCGGA	AGGTAGGTCT	GTAGTAAAAC	GACGGTCCTA
15451		CTTCACCCAC			
	CGCCCCACCT	GAAGTGGGTG	TCGGCGGACT	CGTTGAACAA	CCCGTAGGCG
15501	AAGCGGCAAC	CCTTCCAGGA	GGGCTTTAGG	ATCACCTACG	ATGATCTGGA
	TTCGCCGTTG	GGAAGGTCCT	CCCGAAATCC	TAGTGGATGC	TACTAGACCT
15551	GGGTGGTAAC	ATTCCCGCAC	TGTTGGATGT	GGAÇGCCTAC	CAGGCGAGCT
	CCCACCATTG	TAAGGGCGTG	ACAACCTACA	CCTGCGGATG	GTCCGCTCGA
15601	TGAAAGATGA	CACCGAACAG	GGCGGGGGTG	GCGCAGGCGG	CAGCAACAGC
	ACTTTCTACT	GTGGCTTGTC	CCGCCCCCAC	CGCGTCCGCC	GTCGTTGTCG
15651	AGTGGCAGCG	GCGCGGAAGA	GAACTCCAAC	GCGGCAGCCG	CGGCAATGCA
	TCACCGTCGC	CGCGCCTTCT	CTTGAGGTTG	CGCCGTCGGC	GCCGTTACGT
15701	GCCGGTGGAG	GACATGAACG	ATCATGCCAT	TCGCGGCGAC	ACCTTTGCCA
	CGGCCACCTC	CTGTACTTGC	TAGTACGGTA	AGCGCCGCTG	TGGAAACGGT
15751		GGAGAAGCGC			
	GTGCCCGACT	CCTCTTCGCG	CGACTCCGGC	TTCGTCGCCG	GCTTCGACGG
15801	GCCCCCGCTG	CGCAACCCGA	GGTCGAGAAG	CCTCAGAAGA	AACCGGTGAT
	CGGGGGCGAC	GCGTTGGGCT	CCAGCTCTTC	GGAGTCTTCT	TTGGCCACTA
15851	CAAACCCCTG	ACAGAGGACA	GCAAGAAACG	CAGTTACAAC	CTAATAAGCA
	GTTTGGGGAC	TGTCTCCTGT	CGTTCTTTGC	GTCAATGTTG	GATTATTCGT
15901	ATGACAGCAC				
	TACTGTCGTG	GAAGTGGGTC	ATGGCGTCGA	CCATGGAACG	TATGTTGATG
15951	GGCGACCCTC				
	CCGCTGGGAG	TCTGGCCTTA	GGCGAGTACC	TGGGACGAAA	CGTGAGGACT
160,01	CGTAACCTGC				
	GCATTGGACG	CCGAGCCTCG	TCCAGATGAC	CAGCAACGGT	CTGTACTACG
16051	AAGACCCCGT	GACCTTCCGC	TCCACGCGCC	AGATCAGCAA	CTTTCCGGTG
	TTCTGGGGCA	CTGGAAGGCG	AGGTGCGCGG	TCTAGTCGTT	GAAAGGCCAC

Figure 26 Q

16101	GTGGGCGCCG CACCCGCGGC	A TGTTGCC TLACAACGG	CGTGCACTCC GCACGTGAGG	AAGAGCTTCT TTCTCGAAGA	ACAACG CA TGTTGC CT
16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT	TCCCGAGAAC	CAGATTTTGG	ececeeecee	AGCCCCCACC
	ACTTAGCGAA	AGGGCTCTTG	GTCTAAAACC	cececcecc	TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCECTECEC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TEGCEACECE	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	CCAGACGCCG	CACCTGCCCC	TACGTTTACA	AGGCCCTGGG	CATAGTCTCG
	GGTCTGCGGC	GTGGACGGG	ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
	GGCGCGCAGG	ATAGCTCGGC	GTGAAAAACT	CGTTCGTACA	GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC AACCGCCCCG	CAAGAAGCGC GTTCTTCGCG	TCCGACCAAC AGGCTGGTTG	ACCCAGTGCG TGGGTCACGC	CCTCCCCCCC
16551	CACTACCGCG GTGATGGCGC	CGCCCTGGGG GCGGGACCCC	CGCGCACAAA GCGCGTGTTT	CGCGGCGCGT	CTGGGCGCAC GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	CGCCCACGCC	GCCACCAGTG CGGTGGTCAC	TCCACAGTGG AGGTGTCACC	ACGCGGCCAT TGCGCCGGTA	TCAGACCGTG AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC GTGGCGGCGG	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA ACGGCGGGTT	CCCCCCCCC
16801	CGGCCCTGCT GCCGGGACGA	TAACCGCGCA ATTGGCGCGT	CGTCGCACCG GCAGCGTGGC	GCCGACGGGC	GGCCATGCGG CCGGTACGCC
16851	GCCGCTCGAA CGCCGAGCTT	GGCTGGCCGC	GGGTATTGTC CCCATAACAG	ACTGTGCCCC TGACACGGGG	CCAGGTCCAG GGTCCAGGTC
16901	GCGACGAGCG	GCCGCCGCAG	CAGCCGCGGC	CATTAGTGCT	ATGACTCAGG
	CGCTGCTCGC	CGGCGGCGTC	GTCGGCGCCG	GTAATCACGA	TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG ACCCACGCGC	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC
17001	CTCCCCGTGC	GCACCCGCCC	CCCGCGCAAC	TAGATTGCAA	GAAAAAACTA
	CACGGGCACG	CGTGGGCGGG	GGGCGCGTTG	ATCTAACGTT	CTTTTTTGAT



17051			TGTATCCAGC ACATAGGTCG		
17101			AAAGAAGAGA TTTCTTCTCT		
17151	GAGATCTATG CTCTAGATAC		GAAGGAAGAG CTTCCTTCTC		
17201			AAAAGAAAGA TTTTCTTTCT		
17251			GCTACCGCGC CGATGGCGCG		
17301			TGTTTTGCGA ACAAAACGCT		
17351			CCCGCACCTA GGGCGTGGAT		
17401			CTTGAGCAGG GAACTCGTCC		
17451			TAAGGACATG ATTCCTGTAC		
17501			TAAAGCCCGT ATTTCGGGCA		
17551			GAAAAGCGCG CTTTTCGCGC		
17601			GCTGATGGTA CGACTACCAT		
17651			CCGTGGAACC GGCACCTTGG		
17701			GTGGCGCCGG CACCGCGGCC		
17751			CAGTAGCACC GTCATCGTGG		CCGCCACAGA GGCGGTGTCT
17801	GGGCATGGAG CCCGTACCTC	ACACAAACGT TGTGTTTGCA	CCCCGGTTGC GGGGCCAACG	CTCAGCGGTG GAGTCGCCAC	GCGGATGCCG CGCCTACGGC
17851	CGGTGCACGC GCCACGTCCG	GCTCGCTGCG CCAGCGACGC	GCCGCGTCCA CGGCGCAGGT	AGACCTCTAC TCTGGAGATG	GGAGGTGCAA CCTCCACGTT
17901	ACGGACCCGT TGCCTGGGCA	GGATGTTTCG CCTACAAAGC	CGTTTCAGCC GCAAAGTCGG	9929229999 0029929999	CGCGCCGTTC GCGCGGCAAG
17951	GAGGAAGTAC CTCCTTCATG	GGCGCCGCCA CCGCGGCGGT	GCGCGCTACT CGCGCGATGA	GCCCGAATAT CGGGCTTATA	GCCCTACATC CGGGATGTAG

Figure 265

18001	CTTCCATTGC	GCCTACCCCC	GGCTATCGTG	GCTACACCTA	CCCCCCCAGA
10001	GAAGGTAACG	CATGGGGG	CCGATAGCAC	CGATGTGGAT	GGCGGG
18051	AGACGAGCAA	CTACCCGACG	CCGAACCACC	ACTGGAACCC	GCCGCCGCCG
	TCTGCTCGTT	GATGGGCTGC	GGCTTGGTGG	TGACCTTGGG	CGGCGGCGGC
18101	TCGCCGTCGC	CAGCCCGTGC	TGGCCCCGAT	TTCCGTGCGC	AGGGTGGCTC
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
	CGCTTCCTCC	GTCCTGGGAC	CACGACGGTT	GTCGCGCGAT	GGTGGGGTCG
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
		TCGGCCAGAA			
18251	CCGCCTCCGT	TTCCCGGTGC	CGGGATTCCG	AGGAAGAATG	CACCGTAGGA
		AAGGGCCACG			
18301	GGGGCATGGC	CGGCCACGGC	CTGACGGGCG	GCATGCGTCG	TGCGCACCAC
•		GCCGGTGCCG			
18351	CGGCGGCGGC	GCGCGTCGCA	CCGTCGCATG	CGCGGCGGTA	TCCTGCCCCT
		CGCGCAGCGT			
18401	CCTTATTCCA	CTGATCGCCG	CGGCGATTGG	CGCCGTGCCC	GGAATTGCAT
		GACTAGCGGC			
18451	CCGTGGCCTT	GCAGGCGCAG	AGACACTGAT	TAAAAACAAG	TIGCATGIGG
	_	CGTCCGCGTC			
18501	AAAAATCAAA	ATAAAAAGTC TATTTTTCAG	TGGACTCTCA	CGCTCGCTTG	CACCACATTC
•		AATGGAAGAC			
18551	TATTITGTAG	AATGGAAGAC	MACCAMO AND	CCACACACC	CCCCCCACAC
		CGTTCATGGG			
18601	GGCTCGCGCC	GCAAGTACCC	TTTC & CCCTT	CTATAGCCGT	GGTCGTTATA
	CCGAGCGCGG	GLAAGIACCC	IIIGACCGII	CIMINOCCOI	001001
18651	GAGCGGTGGC	GCCTTCAGCT	GGGGCTCGCT	GTGGAGCGGC	TTAAAAATTA
20032	CTCGCCACCG	CGGAAGTCGA	CCCCGAGCGA	CACCTCGCCG	TAATTTTTAA
			m>m0003>003	N CCCCTCC N N	CAGCAGCACA
18701	TCGGTTCCAC	GCAATTCTTG	TATEGLAGGA	TCCGCACCTT	CTCCTCCTCT
18751	GGCCAGATGC	TGAGGGATAA	GTTGAAAGAG	CAAAATTTCC	AACAAAAGGT
					TTGTTTTCCA
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GCTGGTGGAC	CTGGCCAACC
	CCATCTACCG	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
18851	AGGCAGTGCA	AAATAAGATT	AACAGTAAGC	TTGATCCCCG	CCCTCCCGTA
10071	TCCGTCACGT	TTTATTCTAA	TTGTCATTCG	AACTAGGGGC	GGGAGGGCAT
18901	GAGGAGCCTC	CACCGGCCGT	GGAGACAGTG	TCTCCAGAGG	GGCGTGGCGA
	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

18951	AAAGCGTCCG	CCGACA	GGGAAGAAAC	TCTGGTGACG``	CAAATA G
	TTTCGCAGGC	GCCGGCTGT	CCCTTCTTTG	AGACCACTGC	GTTTATC C
19001	AGCCTCCCTC	GTACGAGGAG	GCACTAAAGC	AAGGCCTGCC	CACCACCCGT
	TCGGAGGGAG	CATGCTCCTC	CGTGATTTCG	TTCCGGACGG	GTGGTGGGCA
19051	CCCATCGCGC	CCATGGCTAC	CGGAGTGCTG	GGCCAGCACA	CACCCGTAAC
	GGGTAGCGCG	GGTACCGATG	GCCTCACGAC	CCGGTCGTGT	GTGGGCATTG
19101	GCTGGACCTG	CCTCCCCCG	CCGACACCCA	GCAGAAACCT	GTGCTGCCAG
	CGACCTGGAC	GGAGGGGGC	GGCTGTGGGT	CGTCTTTGGA	CACGACGGTC
19151	GCCCGACCGC CGGGCTGGCG	CGTTGTTGTA GCAACAACAT	ACCCGTCCTA TGGGCAGGAT	CGGCGCGCAG	CCTGCGCCGC GGACGCGGCG
19201	GCCGCCAGCG	GTCCGCGATC	GTTGCGGCCC	GTAGCCAGTG	GCAACTGGCA
	CGGCGGTCGC	CAGGCGCTAG	CAACGCCGGG	CATCGGTCAC	CGTTGACCGT
19251	AAGCACACTG	AACAGCATCG	TGGGTCTGGG	GGTGCAATCC	CTGAAGCGCC
	TTCGTGTGAC	TTGTCGTAGC	ACCCAGACCC	CCACGTTAGG	GACTTCGCGG
19301	GACGATGCTT	CTGATAGCTA	ACGTGTCGTA	TGTGTGTCAT	GTATGCGTCC
	CTGCTACGAA	GACTATCGAT	TGCACAGCAT	ACACACAGTA	CATACGCAGG
19351	ATGTCGCCGC TACAGCGGCG	CAGAGGAGCT GTCTCCTCGA	GCTGAGCCGC CGACTCGGCG	GCGCGCGCCCG	CTTTCCAAGA GAAAGGTTCT
19401	TGGCTACCCC	TTCGATGATG	CCGCAGTGGT	CTTACATGCA	CATCTCGGGC
	ACCGATGGGG	AAGCTACTAC	GGCGTCACCA	GAATGTACGT	GTAGAGCCCG
19451	CAGGACGCCT	CGGAGTACCT	GAGCCCCGGG	CTGGTGCAGT	TTGCCCGCGC
	GTCCTGCGGA	GCCTCATGGA	CTCGGGGCCC	GACCACGTCA	AACGGGCGCG
19501	CACCGAGACG GTGGCTCTGC	TACTTCAGCC ATGAAGTCGG	TGAATAACAA ACTTATTGTT	GTTTAGAAAC CAAATCTTTG	CCCACGGTGG
19551	CGCCTACGCA	CGACGTGACC	ACAGACCGGT	CCCAGCGTTT	GACGCTGCGG
	GCGGATGCGT	GCTGCACTGG	TGTCTGGCCA	GGGTCGCAAA	CTGCGACGCC
19601	TTCATCCCTG	TGGACCGTGA	GGATACTGCG	TACTCGTACA	AGGCGCGGTT
	AAGTAGGGAC	ACCTGGCACT	CCTATGACGC	ATGAGCATGT	TCCGCGCCAA
19651	CACCCTAGCT	GTGGGTGATA	ACCGTGTGCT	GGACATGGCT	TCCACGTACT
	GTGGGATCGA	CACCCACTAT	TGGCACACGA	CCTGTACCGA	AGGTGCATGA
19701	TTGACATCCG AACTGTAGGC	CGGCGCACGAC	GACAGGGGCC CTGTCCCCGG	CTACTITTAA GATGAAAATT	GCCCTACTCT CGGGATGAGA
19751	GGCACTGCCT	ACAACGCCCT	GGCTCCCAAG	GGTGCCCCAA	ATCCTTGCGA
	CCGTGACGGA	TGTTGCGGGA	CCGAGGGTTC	CCACGGGGTT	TAGGAACGCT
19801	ATGGGATGAA	GCTGCTACTG	CTCTTGAAAT	AAACCTAGAA	GAAGAGGACG
	TACCCTACTT	CGACGATGAC	GAGAACTTTA	TTTGGATCTT	CTTCTCCTGC
19851	ATGACAACGA TACTGTTGCI	AGACGAAGTA TCTGCTTCAT	GACGAGCAAG	CTGAGCAGCA GACTCGTCGT	AAAAACTCAC TTTTTGAGTG

Figure 26 U

19901 .	GTATTTGGGC CATAAACCCG	A TOCCTTA TCCGCGGAAT	TTCTGGTATA AAGACCATAT	AATATTACAA TTATAATGTT	AGGAGG T.T TCCTCCCATA
19951	TCAAATAGGT AGTTTATCCA	GTCGAAGGTC CAGCTTCCAG	AAACACCTAA TTTGTGGATT	ATATGCCGAT TATACGGCTA	AAAACATTTC TTTTGTAAAG
20001	AACCTGAACC TTGGACTTGG	TCAAATAGGA AGTTTATCCT	GAATCTCAGT CTTAGAGTCA	GGTACGAAAC CCATGCTTTG	AGAAATTAAT TCTTTAATTA
20051	GTACGTCGAC	GGAGAGTCCT CCTCTCAGGA	TTTTTTCTGA	TGGGGTTACT	TTGGTACAAT
20101	GCCAAGTATA	GCAAAACCCA CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTAAGAAC
20151	ATTTCGTTGT	AAATGGAAAG TTTACCTTTC	GATCTTTCAG	TTCACCTTTA	CGTTAAAAAG
20201		TCCGTCGGCG	TCCGTTACCA	CTATTGAACT	GAGGATTTCA
20251	CCATAACATG	AGTGAAGATG TCACTTCTAC	ATCTATATCT	TTGGGGTCTG	TGAGTATAAA
20301	GAATGTACGG	CACTATTAAG GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351	GTTAGATACG	CCAACAGGCC GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
20401	ACCAGATTAC	TATTACAACA ATAATGTTGT	CGTGCCCATT	ATACCCACAA	GACCGCCCGG
20451	TTCGTAGCGT	GTTGAATGCT CAACTTACGA	CAACATCTAA	ACCTTCTGTC	TTTGTGTCTC
20501	GAAAGTATGG	AGCTTTTGCT TCGAAAACGA	ACTAAGGTAA	CCACTATCTT	GGTCCATGAA
20551	TTCTATGTGG AAGATACACC	AATCAGGCTG TTAGTCCGAC	TTGACAGCTA AACTGTCGAT	TGATCCAGAT ACTAGGTCTA	GTTAGAATTA CAATCTTAAT
20601	AACTTTTAGT	TGGAACTGAA ACCTTGACTT	CTACTTGAAG	GTTTAATGAC	GAAAGGTGAC
	CCTCCACACT	AATTATGTCT	CTGAGAATGG	TTCCATTTTG	CTAAAACAGG GATTTTGTCC
20701	TCAGGAAAAT AGTCCTTTTA	GGATGGGAAA CCTACCCTTT	AAGATGCTAC TTCTACGATG	AGAATTTTCA TCTTAAAAGT	GATAAAAATG CTATTTTTAC
20751	AAATAAGAGT TTTATTCTCA	TGGAAATAAT ACCTTTATTA	TTTGCCATGG AAACGGTACC	AAATCAATCT TTTAGTTAGA	AAATGCCAAC TTTACGGTTG
20801	CTGTGGAGAA GACACCTCTT	ATTTCCTGTA TAAAGGACAT	CTCCAACATA GAGGTTGTAT	GCGCTGTATT CGCGACATAA	TGCCCGACAA ACGGGCTGTT

Figure 26 V

20851	GCTAAAGTAC	A CCTTCCA	ACGTAAAAAT	TTCTGATÄÄČ	TCAAACACT
	CGATTTCATG	T GAAGGT	TGCATTTTTA	AAGACTATTG	GCTTTCAA
20901	ACGACTACAT	GAACAAGCGA	GTGGTGGCTC	CCGGGCTAGT	GGACTGCTAC
	TGCTGATGTA	CTTGTTCGCT	CACCACCGAG	GGCCCGATCA	CCTGACGATG
20951	ATTAACCTTG	GAGCACGCTG	GTCCCTTGAC	TATATGGACA	ACGTCAACCC
	TAATTGGAAC	CTCGTGCGAC	CAGGGAACTG	ATATACCTGT	TGCAGTTGGG
21001	ATTTAACCAC	CACCGCAATG	CTGGCCTGCG	CTACCGCTCA	ATGTTGCTGG
	TAAATTGGTG	GTGGCGTTAC	GACCGGACGC	GATGGCGAGT	TACAACGACC
21051	GCAATGGTCG	CTATGTGCCC	TTCCACATCC	AGGTGCCTCA	GAAGTTCTTT
	CGTTACCAGC	GATACACGGG	AAGGTGTAGG	TCCACGGAGT	CTTCAAGAAA
21101	GCCATTAAAA	ACCTCCTTCT	CCTGCCGGGC	TCATACACCT	ACGAGTGGAA
	CGGTAATTTT	TGGAGGAAGA	GGACGGCCCG	AGTATGTGGA	TGCTCACCTT
21151	CTTCAGGAAG	GATGTTAACA	TGGTTCTGCA	GAGCTCCCTA	GGAAATGACC
	GAAGTCCTTC	CTACAATTGT	ACCAAGACGT	CTCGAGGGAT	CCTTTACTGG
21201	TAAGGGTTGA	CGGAGCCAGC	ATTAAGTTTG	ATAGCATTTG	CCTTTACGCC
	ATTCCCAACT	GCCTCGGTCG	TAATTCAAAC	TATCGTAAAC	GGAAATGCGG
21251	ACCTTCTTCC	CCATGGCCCA	CAACACCGCC	TCCACGCTTG	AGGCCATGCT
	TGGAAGAAGG	GGTACCGGGT	GTTGTGGCGG	AGGTGCGAAC	TCCGGTACGA
21301	TAGAAACGAC	ACCAACGACC	AGTCCTTTAA	CGACTATCTC	TCCGCCGCCA
	ATCTTTGCTG	TGGTTGCTGG	TCAGGAAATT	GCTGATAGAG	AGGCGGCGGT
21351	ACATGCTCTA	CCCTATACCC	GCCAACGCTA	CCAACGTGCC	CATATCCATC
	TGTACGAGAT	GGGATATGGG	CGGTTGCGAT	GGTTGCACGG	GTATAGGTAG
21401	CCCTCCCGCA	ACTGGGCGGC	TTTCCGCGGC	TGGGCCTTCA	CGCGCCTTAA
	GGGAGGGCGT	TGACCCGCCG	AAAGGCGCCG	ACCCGGAAGT	GCGCGGAATT
21451	GACTAAGGAA	ACCCCATCAC	TGGGCTCGGG	CTACGACCCT	TATTACACCT
	CTGATTCCTT	TGGGGTAGTG	ACCCGAGCCC	GATGCTGGGA	ATAATGTGGA
21501	ACTCTGGCTC	TATACCCTAC	CTAGATGGAA	CCTTTTACCT	CAACCACACC
	TGAGACCGAG	ATATGGGATG	GATCTACCTT	GGAAAATGGA	GTTGGTGTGG
21551	TTTAAGAAGG AAATTCTTCC	TGGCCATTAC ACCGGTAATG	CTTTGACTCT GAAACTGAGA	TCTGTCAGCT AGACAGTCGA	GGCCTGGCAA
21601	TGACCGCCTG	CTTACCCCCA	ACGAGTITGA	AATTAAGCGC	TCAGTTGACG
	ACTGGCGGAC	GAATGGGGGT	TGCTCAAACT	TTAATTCGCG	AGTCAACTGC
21651	GGGAGGGTTA	CAACGTTGCC	CAGTGTAACA	TGACCAAAGA	CTGGTTCCTG
	CCCTCCCAAT	GTTGCAACGG	GTCACATTGT	ACTGGTTTCT	GACCAAGGAC
21701	GTACAAATGC CATGTTTACG	TAGCTAACTA ATCGATTGAT	TAACATTGGC	TACCAGGGCT	TCTATATCCC AGATATAGGG
21751	AGAGAGCTAC TCTCTCGATG	AAGGACCGCA TTCCTGGCGI	TGTACTCCTT ACATGAGGA	CTTTAGAAAC A GAAATCTTTG	TTCCAGCCCA AAGGTCGGGT

Figure 26 W

21801	TGAGCCGTCA ACTCGGCAGT	COTCACCTA	GATACÎAAAT CTATGATTTA	ACAAGGACTAS TGTTCCTGAT	CCAACACTIC GGTTGT
21851	GGCATCCTAC CCGTAGGATG	ACCAACACAA TGGTTGTGTT	CAACTCTGGA GTTGAGACCT	TTTGTTGGCT AAACAACCGA	ACCTTGCCCC TGGAACGGGG
21901	GTGGTACGCG	CTTCCTGTCC	GGATGGGACG	TAACTTCCCC ATTGAAGGGG	ATAGGCGAAT
21951	ATCCGTTCTG	GCGTCAACTG	TCGTAATGGG	AGAAAAAGTT TCTTTTTCAA	AGAAACGCTA
22001	GCGTGGGAAA	CCGCGTAGGG	TAAGAGGTCA	AACTTTATGT TTGAAATACA	GGTACCCGCG
22051	TGAGTGTCTG	GACCCGGTTT	TGGAAGAGAT	CGCCAACTCC GCGGTTGAGG	CGGGTGCGCG
22101	ATCTGTACTG	AAAACTCCAC	CTAGGGTACC	ACGAGCCCAC TGCTCGGGTG	GGAAGAAATA
22151	CAAAACAAAC	TTCAGAAACT	GCACCAGGCA	GTGCACCAGC CACGTGGTCG	GCGTGGCGCC
22201	GCAGTAGCTT	TGGCACATGG	ACGCGTGCGG	CTTCTCGGCC GAAGAGCCGG	CCGTTGCGGT
22251	GTTGTATTTC	TTCGTTCGTT	GTAGTTGTTG	AGCTGCCGCC TCGACGGCGG	TACCCGAGGT
22301	CACTCGTCCT	TGACTTTCGG	TAACAGTTTC	ATCTTGGTTG TAGAACCAAC	ACCCGGTATA
22351	AAAAACCCGT	GGATACTGTT	CGCGAAAGGT	GGCTTTGTTT CCGAAACAAA	GAGGTGTGTT
22401	CGAGCGGACG	CGGTATCAGT	TATGCCGGCC	TCGCGAGACT AGCGCTCTGA	CCCCCGCATG
22451	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	CAAAAACATG GTTTTTGTAC	GATGGAGAAA
22501	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	AAGCAGGTTT TTCGTCCAAA	TGGTCAAACT
		GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
		CCTTTTCAGG	TGGGTTTCGC	ATGTCCCCGG	GTTGAGCCGG
		ATAAGACGAC	GTACAAAGAG	GTGCGGAAAC	GGTTGACCGG
22701	CCAAACTCCC GGTTTGAGGG	ATGGATCACA TACCTAGTGT	ACCCCACCAT TGGGGTGGTA	GAACCTTATT CTTGGAATAA	ACCGGGGTAC TGGCCCCATG

Figure 26 X

22751	GGTTGAGGTA.	CTTGTCA	GGGGTCCATG	TCGGGTGGGY	CGCAGC
22801	CAGGAACAGC	TCTACAGCTT	CCTGGAGCGC	CACTCGCCCT	ACTTCCGCAG
	GTCCTTGTCG	AGATGTCGAA	GGACCTCGCG	GTGAGCGGGA	TGAAGGCGTC
22851	CCACAGTGCG	CAGATTAGGA	GCGCCACTTC	TTTTTGTCAC	TTGAAAAACA
	GGTGTCACGC	GTCTAATCCT	CGCGGTGAAG	AAAAACAGTG	AACTTTTTGT
22901	TGTAAAAATA	ATGTACTAGA	GACACTTTCA	ATAAAGGCAA	ATGCTTTTAT
	ACATTTTTAT	TACATGATCT	CTGTGAAAGT	TATTTCCGTT	TACGAAAATA
22951	TTGTACACTC	TCGGGTGATT	ATTTACCCCC	ACCCTTGCCG	TCTGCGCCGT
	AACATGTGAG	AGCCCACTAA	TAAATGGGGG	TGGGAACGGC	AGACGCGGCA
23001	TTAAAAATCA	AAGGGGTTCT	GCCGCGCATC	GCTATGCGCC	ACTGGCAGGG
	AATTTTTAGT	TTCCCCAAGA	CGGCGCGTAG	CGATACGCGG	TGACCGTCCC
23051	ACACGTTGCG	ATACTGGTGT	TTAGTGCTCC	ACTTAAACTC	AGGCACAACC
	TGTGCAACGC	TATGACCACA	AATCACGAGG	TGAATTTGAG	TCCGTGTTGG
23101	ATCCGCGGCA	GCTCGGTGAA	GTTTTCACTC	CACAGGCTGC	GCACCATCAC
	TAGGCGCCGT	CGAGCCACTT	CAAAAGTGAG	GTGTCCGACG	CGTGGTAGTG
23151	CAACGCGTTT	AGCAGGTCGG	GCGCCGATAT	CTTGAAGTCG	CAGTTGGGGC
	GTTGCGCAAA	TCGTCCAGCC	CGCGGCTATA	GAACTTCAGC	GTCAACCCCG
23201	CTCCGCCCTG	CGCGCGCGAG	TTGCGATACA	CAGGGTTGCA	GCACTGGAAC
	GAGGCGGGAC	GCGCGCGCTC	AACGCTATGT	GTCCCAACGT	CGTGACCTTG
23251	ACTATCAGCG	CCGGGTGGTG	CACGCTGGCC	AGCACGCTCT	TGTCGGAGAT
	TGATAGTCGC	GGCCCACCAC	GTGCGACCGG	TCGTGCGAGA	ACAGCCTCTA
23301	CAGATCCGCG	TCCAGGTCCT	CCGCGTTGCT	CAGGGCGAAC	GGAGTCAACT
	GTCTAGGCGC	AGGTCCAGGA	GGCGCAACGA	GTCCCGCTTG	CCTCAGTTGA
23351	TTGGTAGCTG	CCTTCCCAAA	AAGGGCGCGT	GCCCAGGCTT	TGAGTTGCAC
	AACCATCGAC	GGAAGGGTTT	TTCCCGCGCA	CGGGTCCGAA	ACTCAACGTG
23401	TCGCACCGTA	GTGGCATCAA	AAGGTGACCG	TGCCCGGTCT	GGGCGTTAGG
	AGCGTGGCAT	CACCGTAGTT	TTCCACTGGC	ACGGGCCAGA	CCCGCAATCC
23451	ATACAGCGCC	TGCATAAAAG	CCTTGATCTC	CTTAAAAGCC	ACCTGAGCCT
	TATGTCGCGG	ACGTATTTTC	GGAACTAGAC	GAATTTTCGG	TGGACTCGGA
23501	TTGCGCCTTC	AGAGAAGAAC	ATGCCGCAAC	ACTTGCCGGA	AAACTGATTG
	AACGCGGAAG	TCTCTTCTTC	TACGGCGTTC	TGAACGGCCT	TTTGACTAAC
23551	GCCGGACAGG CGGCCTGTCC	CCGCGTCGTC GGCGCAGCAC	CACGCAGCAC CGTGCGTCGTC	CTTGCGTCGG GAACGCAGCC	TGTTGGAGAT
23601	CTGCACCACA GACGTGGTGT	TTTCGGCCCC	C ACCGGTTCT	r cacgatettg A gtgetagaac	GCCTTGCTAG CGGAACGATC
23651	ACTGCTCCTT TGACGAGGA	CAGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG	TGCCCGTTT	r CGCTCGTCAC A GCGAGCAGTG	ATCCATTTCA TAGGTAAAGT

France 26 Y

23701	ATCACGTGCT	CS TATTTAT	CATAATGCTT	CCGTGTAGAC	ACTTAA CC
	TAGTGCACGA	GGAATAAATA	GTATTACGAA	GGCACATCTG	TGAATTCGAG
23751	GCCTTCGATC	TCAGCGCAGC	GGTGCAGCCA	CAACGCGCAG	CCCGTGGGCT
	CGGAAGCTAG	AGTCGCGTCG	CCACGTCGGT	GTTGCGCGTC	GGGCACCCGA
23801	CGTGATGCTT	GTAGGTCACC	TCTGCAAACG	ACTGCAGGTA	CGCCTGCAGG
	GCACTACGAA	CATCCAGTGG	AGACGTTTGC	TGACGTCCAT	GCGGACGTCC
23851	AATCGCCCCA	TCATCGTCAC	AAAGGTCTTG	TTGCTGGTGA	AGGTCAGCTG
	TTAGCGGGGT	AGTAGCAGTG	TTTCCAGAAC	AACGACCACT	TCCAGTCGAC
23901	CAACCCGCGG	TGCTCCTCGT	TCAGCCAGGT	CTTGCATACG	GCCGCCAGAG
	GTTGGGCGCC	ACGAGGAGCA	AGTCGGTCCA	GAACGTATGC	CGGCGGTCTC
23951	CTTCCACTTG	GTCAGGCAGT	AGTTTGAAGT	TCGCCTTTAG	ATCGTTATCC
	GAAGGTGAAC	CAGTCCGTCA	TCAAACTTCA	AGCGGAAATC	TAGCAATAGG
24001	ACGTGGTACT TGCACCATGA	TGTCCATCAG ACAGGTAGTC	CGCGCGCGCA TDCDCDCDCDC	GCCTCCATGC CGGAGGTACG	CCTTCTCCCA
24051	CGCAGACACG	ATCGGCACAC	TCAGCGGGTT	CATCACCGTA	ATTTCACTTT
	GCGTCTGTGC	TAGCCGTGTG	AGTCGCCCAA	GTAGTGGCAT	TAAAGTGAAA
24101	CCGCTTCGCT	GGGCTCTTCC	TCTTCCTCTT	GCGTCCGCAT	ACCACGCGCC
	GGCGAAGCGA	CCCGAGAAGG	AGAAGGAGAA	CGCAGGCGTA	TGGTGCGCGG
24151	ACTGGGTCGT	CTTCATTCAG	CCGCCGCACT	GTGCGCTTAC	CTCCTTTGCC
	TGACCCAGCA	GAAGTAAGTC	GGCGGCGTGA	CACGCGAATG	GAGGAAACGG
24201	ATGCTTGATT	AGCACCGGTG	GGTTGCTGAA	ACCCACCATT	TGTAGCGCCA
	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	ACATCGCGGT
24251	CATCTTCTCT	TTCTTCCTCG	CTGTCCACGA	TTACCTCTGG	TGATGGCGGG
	GTAGAAGAGA	AAGAAGGAGC	GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301	CGCTCGGGCT GCGAGCCCGA	TGGGAGAAGG ACCCTCTTCC	GCGCTTCTTT	TTCTTCTTGG AAGAAGAACC	GCGCAATGGC CGCGTTACCG
24351	CAAATCCGCC GTTTAGGCGG	GCCGAGGTCG CGGCTCCAGC	ATGGCCGCGG TACCGGCGCC	GCTGGGTGTG CGACCCACAC	CGCGGCACCA
24401	GCGCGTCTTG	TGATGAGTCT	TCCTCGTCCT	CGGACTCGAT	ACGCCGCCTC
	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	GCCTGAGCTA	TGCGGCGGAG
24451	ATCCGCTTTT TAGGCGAAAA	TTGGGGGCGC AACCCCCGCG	CCGGGGAGGC	CCGCCGCTGC	CCCTGCCCCT
24501	CGACACGTCC GCTGTGCAGG	TCCATGGTTG AGGTACCAAC	GGGGACGTCG CCCCTGCAGC	CGCCGCACCG	CGTCCGCGCT
24551	CGGGGGTGGT	TTCGCGCTGC	TCCTCTTCCC	GACTGGCCAT	TTCCTTCTCC
	GCCCCACCA	AAGCGCGACG	AGGAGAAGGG	CTGACCGGTA	AAGGAAGAGG
24601	TATAGGCAGA	AAAAGATCAT	GGAGTCAGTC	GAGAAGAAGG	ACAGCCTAAC
	ATATCCGTCT	TTTTCTAGTA	CCTCAGTCAG	CTCTTCTTCC	TGTCGGATTG

Figure 262

24651	CGCCCCCTCT GCGGGGGAGA	TTCGCCA CTCAAGCGGT	CCACCGCCTC	CACCGATGCC GTGGCTACGG	GUCAAC CGGTTGCGCG
24701	CTACCACCTT	CCCCGTCGAG	GCACCCCCGC	TTGAGGAGGA	GGAAGTGATT
24,02	GATGGTGGAA	GGGGCAGCTC	CGTGGGGGCG	AACTCCTCCT	CCTTCACTAA
24751	ATCGAGCAGG	ACCCAGGTTT	TGTAAGCGAA	GACGACGAGG	ACCGCTCAGT
	TAGCTCGTCC	TGGGTCCAAA	ACATTCGCTT	CTGCTGCTCC	TGGCGAGTCA
24801	ACCAACAGAG	GATAAAAAGC	AAGACCAGGA	CAACGCAGAG	GCAAACGAGG
	TGGTTGTCTC	CTATTTTTCG	TTCTGGTCCT	GTTGCGTCTC	CGTTTGCTCC
24851	AACAAGTCGG	GCGGGGGGAC	GAAAGGCATG	GCGACTACCT	AGATGTGGGA
2100-	TTGTTCAGCC	CCCCCCTG	CTTTCCGTAC	CGCTGATGGA	TCTACACCCT
24901	GACGACGTGC	TGTTGAAGCA	TCTGCAGCGC	CAGTGCGCCA	TTATCTGCGA
	CTGCTGCACG	ACAACTTCGT	AGACGTCGCG	GTCACGCGGT	AATAGACGCT
24951	CGCGTTGCAA	GAGCGCAGCG	ATGTGCCCCT	CGCCATAGCG	GATGTCAGCC
	GCGCAACGTT	CTCGCGTCGC	TACACGGGGA	GCGGTATCGC	CTACAGTCGG
25001	TTGCCTACGA	ACGCCACCTA	TTCTCACCGC	GCGTACCCCC	CAAACGCCAA
			AAGAGTGGCG		
25051	GAAAACGGCA	CATGCGAGCC	CAACCCGCGC	CTCAACTTCT	ACCCCGTATT
			CTTGGGCGCG		
25101	TGCCGTGCCA	GAGGTGCTTG	CCACCTATCA	CATCTTTTTC	CAAAACTGCA
			GGTGGATAGT		
25151	AGATACCCCT	ATCCTGCCGT	GCCAACCGCA	GCCGAGCGGA	CAAGCAGCTG
			CGGTTGGCGT	•	
25201	GCCTTGCGGC	AGGGCGCTGT	CATACCTGAT	ATCGCCTCGC	TCAACGAAGT
	•		GTATGGACTA		
25251	GCCAAAAATC	TTTGAGGGTC	TTGGACGCGA AACCTGCGCT	CCACAMACCCC	CCCCCTTTCC
					AGTGTTGGTG
25301	CTCTGCAACA	GGAAAACAGC	CTTTTACTTT	CACTCACACAC	TCACAACCAC
					GCAGCATCGA
25351	GAACTCGAGG	G1GACAACGC	CCCCCATCCC	CANCAMMAN	CGTCGTAGCT
					AAGGTCATGA
25401	GGTCACCCAC	THECCTACC	COCCOCALIAN	CCIACCCCC	TTCCAGTACT
25451	GCACAGTCAT	GAGIGAGUIG	MYCCYCCCC	. GIGCGCWGCC	CCTGGAGAGG GGACCTCTCC
	CGTGTCAGTA	CTCACTCGAC	. TAGLAUGUGG	- MCGCGICGG	- AGMICTICACE
25525	0300033300	י הכראאנאארא	אאראניאניטניאני	GGCCTACCCG	CAGTTGGCGA
25501	CHACCHAMI'I	י פרשעפעערע י פרשעפעערע	TTGTCTCCTC	CCGGATGGG	GTCAACCGCT
	•				
25551	CGAGCAGCTA	GCGCGCTGGC	TTCAAACGC	CGAGCCTGC	GACTTGGAGG
	GCTCGTCGAT	CGCGCGACCG	AAGTTTGCGC	CCTCGGACG	CTGAACCTCC

7 igure 26 AA

25601	AGCGACGCAA	A ATGATG	GCCGCAGTGC	TCGTTACCGT	GGAGCTAG
	TCGCTGCGTT	TGATTACTAC	CGGCGTCACG	AGCAATGGCA	CCTCGAACTC
25651	TGCATGCAGC	GGTTCTTTGC	TGACCCGGAG	ATGCAGCGCA	AGCTAGAGGA
	ACGTACGTCG	CCAAGAAACG	ACTGGGCCTC	TACGTCGCGT	TCGATCTCCT
25701	AACATTGCAC	TACACCTTTC	GACAGGGCTA	CGTACGCCAG	GCCTGCAAGA
	TTGTAACGTG	ATGTGGAAAG	CTGTCCCGAT	GCATGCGGTC	CGGACGTTCT
25751	TCTCCAACGT	GGAGCTCTGC	AACCTGGTCT	CCTACCTTGG	AATTTTGCAC
	AGAGGTTGCA	CCTCGAGACG	TTGGACCAGA	GGATGGAACC	TTAAAACGTG
25801	GAAAACCGCC	TTGGGCAAAA	CGTGCTTCAT	TCCACGCTCA	AGGGCGAGGC
	CTTTTGGCGG	AACCCGTTTT	GCACGAAGTA	AGGTGCGAGT	TCCCGCTCCG
25851	GCGCCGCGAC	TACGTCCGCG	ACTGCGTTTA	CTTATTTCTA	TGCTACACCT
	CGCGGCGCTG	ATGCAGGCGC	TGACGCAAAT	GAATAAAGAT	ACGATGTGGA
25901	GGCAGACGGC	CATGGGCGTT	TGGCAGCAGT	GCTTGGAGGA	GTGCAACCTC
	CCGTCTGCCG	GTACCCGCAA	ACCGTCGTCA	CGAACCTCCT	CACGTTGGAG
25951	AAGGAGCTGC	AGAAACTGCT	AAAGCAAAAC	TTGAAGGACC	TATGGACGGC
	TTCCTCGACG	TCTTTGACGA	TTTCGTTTTG	AACTTCCTGG	ATACCTGCCG
26001	CTTCAACGAG	CGCTCCGTGG	CCGCGCACCT	GGCGGACATC	ATTTTCCCCG
	GAAGTTGCTC	GCGAGGCACC	GGCGCGTGGA	CCGCCTGTAG	TAAAAGGGGC
26051	AACGCCTGCT	TAAAACCCTG	CAACAGGGTC	TGCCAGACTT	CACCAGTCAA
	TTGCGGACGA	ATTTTGGGAC	GTTGTCCCAG	ACGGTCTGAA	GTGGTCAGTT
26101	AGCATGTTGC	AGAACTTTAG	GAACTTTATC	CTAGAGCGCT	CAGGAATCTT
	TCGTACAACG	TCTTGAAATC	CTTGAAATAG	GATCTCGCGA	GTCCTTAGAA
26151	CGGGCGGTGG	TGCTGTGCAC ACGACACGTG	AAGGATCGCT	GAAACACGGG	TAATTCATGG
26201	GCGAATGCCC	TCCGCCGCTT	TGGGGCCACT	GCTACCTTCT	GCAGCTAGCC
	CGCTTACGGG	AGGCGGCGAA	ACCCCGGTGA	CGATGGAAGA	CGTCGATCGG
26251	AACTACCTTG	CCTACCACTC	TGACATAATG	GAAGACGTGA	GCGGTGACGG
	TTGATGGAAC	GGATGGTGAG	ACTGTATTAC	CTTCTGCACT	CGCCACTGCC
26301	TCTACTGGAG AGATGACCTC	TGTCACTGTC ACAGTGACAG	GCTGCAACCT CGACGTTGGA	ATGCACCCCG TACGTGGGGC	CACCGCTCCC
26351	TGGTTTGCAA ACCAAACGTT	TTCGCAGCTG AAGCGTCGAC	CTTAACGAAA GAATTGCTTT	GTCAAATTAT CAGTTTAATA	CGGTACCTTT
26401	GAGCTGCAGG CTCGACGTCC	GTCCCTCGCC CAGGGAGCGG	TGACGAAAAG ACTGCTTTTC	TCCGCGGCTC AGGCGCCGAG	CCCCAACTT
26451	ACTCACTCCG	GGGCTGTGGA	CGTCGGCTTA	CCTTCGCAAA	TTTGTACCTG
	TGAGTGAGGC	CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501	AGGACTACCA	CGCCCACGAG	ATTAGGTTCT	ACGAAGACCA	ATCCCGCCCG
	TCCTGATGGT	GCGGGTGCTC	TAATCCAAGA	TGCTTCTGGT	TAGGGCGGGC

Figure 26 AB

26551		A TACCGC TCGAATGGCG			
26601		GCCATCAACA CGGTAGTTGT			
26651	GACGGGGGGT CTGCCCCCCA	TTACTTGGAC AATGAACCTG			
26701		CGCAGCCCTA GCGTCGGGAT			
26751		CAAAAAGAAG GTTTTTCTTC			
26801		GGGACAGTCA CCCTGTCAGT			
26851		GAAGACTGGG CTTCTGACCC			
26901	AAGAGGTGTC TTCTCCACAG	AGACGAAACA TCTGCTTTGT			
26951		AATCGGCAAC TTAGCCGTTG			
27001		CCGGCACTGC			
27051		CAGGGCCGGT GTCCCGGCCA			
27101					
		AGCGCCAAGG	CTACCGCTCA GATGGCGAGT		
27151	CTCGTTGTTG CATAGTTGCT		GATGGCGAGT ACTGTGGGGG	ACCGCGCCCG CAACATCTCC	TGTTCTTGCG TTCGCCCGCC
27151 27201	CTCGTTGTTG  CATAGTTGCT GTATCAACGA  GCTTTCTTCT	TCGCGGTTCC TGCTTGCAAG ACGAACGTTC CTACCATCAC	GATGGCGAGT ACTGTGGGGG TGACACCCCC GGCCTGGCCT	ACCGCGCCCG CAACATCTCC GTTGTAGAGG TCCCCCGTAA	TGTTCTTGCG TTCGCCCGCC
	CTCGTTGTTG CATAGTTGCT GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC	TCGCGGTTCC TGCTTGCAAG ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG	GATGGCGAGT ACTGTGGGGG TGACACCCCC GGCGTGGCCT CCGCACCGGA CCCATACTGC	ACCGCGCCCG CAACATCTCC GTTGTAGAGG TCCCCCGTAA AGGGGGGCATT ACCGGCGCCA	TGTTCTTGCG  TTCGCCCGCC AAGCGGGCGG  CATCCTGCAT
27201 27251	CTCGTTGTTG CATAGTTGCT GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC ATGATGGCAG CAGCAGCGGC	TCGCGGTTCC TGCTTGCAAG ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG TAGAGATGTC CACACAGAAG	GATGGCGAGT ACTGTGGGGG TGACACCCCC GGCGTGGCCT CCGCACCGGA CCCATACTGC GGGTATGACG CAAAGGCGAC	ACCGCGCCCG CAACATCTCC GTTGTAGAGG TCCCCGTAA AGGGGGCATT ACCGGCGCCAT TGGCCGCCGT CGGATAGCAA	TGTTCTTGCG  TTCGCCCGCC AAGCGGGCGG  CATCCTGCAT GTAGGACGTA  GCGGCAGCAA
27201 27251 27301	CTCGTTGTTG CATAGTTGCT GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC ATGATGGCAG CAGCAGCGGC GTCGTCGCCG AAGCCCAAGA	TCGCGGTTCC TGCTTGCAAG ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG TAGAGATGTC CACACAGAAG GTGTGTCTTC	GATGGCGAGT ACTGTGGGGG TGACACCCCC GGCGTGGCCT CCGCACCGGA CCCATACTGC GGGTATGACG CAAAGGCGAC GTTTCCGCTG GGCGGCAGCA	ACCGCGCCCG CAACATCTCC GTTGTAGAGG TCCCCGTAA AGGGGGCATT ACCGGCGCCA TGGCCGCCGT CGGATAGCAA GCCTATCGTT GCAGGAGGAG	TGTTCTTGCG  TTCGCCCGCC AAGCGGGCGG  CATCCTGCAT GTAGGACGTA  GCGCCAGCAA CGCCGTCGTT  GACTCTGACA CTGAGACTGT
27201 27251 27301 27351	CTCGTTGTTG CATAGTTGCT GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC ATGATGGCAG CAGCAGCGGC GTCGTCGCCG AAGCCCAAGA TTCGGGTTCT TCTGGCGCCC	TCGCGGTTCC TGCTTGCAAG ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG TAGAGATGTC CACACAGAAG GTGTGTCTTC AATCCACAGC TTAGGTGTCG AACGAACCCG	GATGGCGAGT ACTGTGGGGG TGACACCCCC GGCGTGGCCT CCGCACCGGA CCCATACTGC GGGTATGACG CAAAGGCGAC GTTTCCGCTG GGCGGCAGCA CCGCCGTCGT TATCGACCCG	ACCGCGCCCG CAACATCTCC GTTGTAGAGG TCCCCCGTAA AGGGGGCATT ACCGGCGGCA TGGCCGCCGT CGGATAGCAA GCCTATCGTT GCAGGAGGAG CGTCCTCCTC	TGTTCTTGCG  TTCGCCCGCC AAGCGGGCGG  CATCCTGCAT GTAGGACGTA  GCGGCAGCAA CGCCGTCGTT  GACTCTGACA CTGAGACTGT  GAGCGCTGCG CTCGCGACGC

Figure 26 AC

27501	GACTTTTATT	CAGGTC	AGACGCTAGG	GAGTGGGCGT	CGACGGACAT
27551	TCACAAAAGC	GAAGATCAGC	TTCGGCGCAC	GCTGGAAGAC	GCGGAGGCTC
	AGTGTTTTCG	CTTCTAGTCG	AAGCCGCGTG	CGACCTTCTG	CGCCTCCGAG
27601	TCTTCAGTAA	ATACTGCGCGC	CTGACTCTTA	AGGACTAGTT	TCGCGCCCTT
	AGAAGTCATT	TATGACGCGC	GACTGAGAAT	TCCTGATCAA	AGCGCGGGAA
27651	TCTCAAATTT	AAGCGCGAAA	ACTACGTCAT	CTCCAGCGGC	CACACCCGGC
	AGAGTTTAAA	TTCGCGCTTT	TGATGCAGTA	GAGGTCGCCG	GTGTGGGCCG
27701	CGGTCGTGGA	GTTGTCAGCG CAACAGTCGC	GGTAATACTC	GTTCCTTTAA	GGGTGCGGGA
27751	ACATGTGGAG	TTACCAGCCA	CAAATGGGAC	TTGCGGCTGG	AGCTGCCCAA
	TGTACACCTC	AATGGTCGGT	GTTTACCCTG	AACGCCGACC	TCGACGGGTT
27801	CTGATGAGTT	CCCGAATAAA GGGCTTATTT	GATGTACTCG	CGCCCTGGGG	TGTACTATAG
27851	CCGGGTCAAC	GGAATACGCG	CCCACCGAAA	CCGAATTCTC	CTGGAACAGG
	GGCCCAGTTG	CCTTATGCGC	GGGTGGCTTT	GGCTTAAGAG	GACCTTGTCC
27901	CGGCTATTAC	CACCACACCT	CGTAATAACC	TTAATCCCCG	TAGTTGGCCC
	GCCGATAATG	GTGGTGTGGA	GCATTATTGG	AATTAGGGGC	ATCAACCGGG
27951	GCTGCCCTGG	TGTACCAGGA	AAGTCCCGCT	CCCACCACTG	TGGTACTTCC
	CGACGGGACC	ACATGGTCCT	TTCAGGGCGA	GGGTGGTGAC	ACCATGAAGG
28001	CAGAGACGCC	CAGGCCGAAG	TTCAGATGAC	TAACTCAGGG	GCGCAGCTTG
	GTCTCTGCGG	GTCCGGCTTC	AAGTCTACTG	ATTGAGTCCC	CGCGTCGAAC
28051	CGGGCGGCTT GCCCGCCGAA	TCGTCACAGG AGCAGTGTCC	GTGCGGTCGC	CCGGGCAGGG	TATAACTCAC ATATTGAGTG
28101	CTGACAATCA	GAGGGCGAGG	TATTCAGCTC	AACGACGAGT	CGGTGAGCTC
	GACTGTTAGT	CTCCCGCTCC	ATAAGTCGAG	TTGCTGCTCA	GCCACTCGAG
28151	CTCGCTTGGT GAGCGAACCA	CTCCGTCCGG GAGGCAGGCC	ACGGGACATT TGCCCTGTAA	TCAGATCGGC AGTCTAGCCG	00000000000000000000000000000000000000
28201	GCTCTTCATT	CACGCCTCGT	CAGGCAATCC	TAACTCTGCA	GACCTCGTCC
	CGAGAAGTAA	GTGCGGAGCA	GTCCGTTAGG	ATTGAGACGT	CTGGAGCAGG
28251	TCTGAGCCGC	GCTCTGGAGG	CATTGGAACT	CTGCAATTTA	TTGAGGAGTT
	AGACTCGGCG	CGAGACCTCC	GTAACCTTGA	GACGTTAAAT	AACTCCTCAA
28301	TGTGCCATCG	GTCTACTTTA	ACCCCTTCTC	GGGACCTCCC	GGCCACTATC
	ACACGGTAGC	CAGATGAAAT	TGGGGAAGAG	CCCTGGAGGG	CCGGTGATAG
28351	CGGATCAATT	TATTCCTAAC	TTTGACGCGG	TAAAGGACTC	GGCGGACGGC
	GCCTAGTTAA	ATAAGGATTG	AAACTGCGCC	ATTTCCTGAG	CCGCCTGCCG
28401	TACGACTGAA ATGCTGACTT	TGTTAAGTGG	AGAGGCAGAG TCTCCGTCTC	CAACTGCGCC GTTGACGCGG	TGAAACACCT ACTTTGTGGA

Figure 26 AD

28451	GGTCCACTGT CCAGGTGACA		AGTGCTTTGC TCACGAAACG		
28501			GATCATATCG CTAGTATAGC		
28551			GCTTGCCCGT CGAACGGGCA		
28601			AGCGGGACAG TCGCCCTGTC		
28651			CCTGGATTAC GGACCTAATG		
28701			ATACAGAAAT TATGTCTTTA		
28751			ACCGTCTTCA TGGCAGAAGT		-
28801			TAACATCTCT ATTGTAGAGA		-
28851			GTCTACGAGA CAGATGCTCT		
28901	ACTCCATCAG	AAAAAACACC	<b>ACCCTACCTATA</b>	CCTCCCCCC	ACCURACE ACC
			TGGGAGGAAT		
28951	TGAGGTAGTC GCGTCACCGG	TTTTTTGTGG CCGCTGCACC		GGACGGCCCT CCTGACCGTA	TGCATGCTCA AACCAGACTT
28951 29001	TGAGGTAGTC GCGTCACCGG CGCAGTGGCC TTTCCGGACA	TTTTTTGTGG CCGCTGCACC GGCGACGTGG GACCTCAATA	TGGGAGGAAT ACACCTACCG	GGACGGCCCT CCTGACCGTA GGACTGGCAT CCAGAACAGG	TGCATGCTCA  AACCAGACTT TTGGTCTGAA  AGGTGAGCTT
	TGAGGTAGTC GCGTCACCGG CGCAGTGGCC TTTCCGGACA AAAGGCCTGT AGAAAACCCT	TTTTTTGTGG CCGCTGCACC GGCGACGTGG GACCTCAATA CTGGAGTTAT TAGGGTATTA	TGGGAGGAAT ACACCTACCG TGTGGATGGC ACTCTGTTTA	GGACGGCCTT CCTGACCGTA GGACTGGCAT CCAGAACAGG GGTCTTGTCC GCAGCTACTG	TGCATGCTCA  AACCAGACTT TTGGTCTGAA  AGGTGAGCTT TCCACTCGAA  TGGGGTTTAT
29001	TGAGGTAGTC GCGTCACCGG CGCAGTGGCC TTTCCGGACA AAAGGCCTGT AGAAAACCCT TCTTTTGGGA GAACAATTCA	TTTTTTGTGG CCGCTGCACC GGCGACGTGG GACCTCAATA CTGGAGTTAT TAGGGTATTA ATCCCATAAT AGCAACTCTA	TGGGAGGAAT ACACCTACCG TGTGGATGGC ACTCTGTTTA TGAGACAAAT GGCCAAAGGC	GGACGGCCT CCTGACCGTA GGACTGGCAT CCAGAACAGG GGTCTTGTCC GCAGCTACTG CGTCGATGAC TAATTCAGGT	TGCATGCTCA  AACCAGACTT TTGGTCTGAA  AGGTGAGCTT TCCACTCGAA  TGGGGTTTAT ACCCCAAATA  TTCTCTAGAA
29001 29051	TGAGGTAGTC GCGTCACCGG CGCAGTGGCC TTTCCGGACA AAAGGCCTGT AGAAAACCCT TCTTTTGGGA GAACAATTCA CTTGTTAAGT TCGGGGTTGG	CCGCTGCACC GGCGACGTGG GACCTCAATA CTGGAGTTAT TAGGGTATTA ATCCCATAAT AGCAACTCTA TCGTTGAGAT GGTTATTCTC	TGGGAGGAAT ACACCTACCG TGTGGATGGC ACTCTGTTTA TGAGACAAAT GGCCAAAGGC CCGGTTTCCG CGGGCTATTC	GGACGGCCT CCTGACCGTA GGACTGGCAT CCAGAACAGG GGTCTTGTCC GCAGCTACTG CGTCGATGAC TAATTCAGGT ATTAAGTCCA TTCTCTTTAT	AACCAGACTT TTGGTCTGAA AGGTGAGCTT TCCACTCGAA TGGGGTTTAT ACCCCAAATA TTCTCTAGAA AAGAGATCTT TCTTATACTA
29001 29051 29101 29151	TGAGGTAGTC  GCGTCACCGG CGCAGTGGCC  TTTCCGGACA AAAGGCCTGT  AGAAAACCCT TCTTTTGGGA  GAACAATTCA CTTGTTAAGT  TCGGGGTTGG AGCCCCAACC  ACGCTTCTCT	CCGCTGCACC GGCGACGTGG GACCTCAATA CTGGAGTTAT TAGGGTATTA ATCCCATAAT AGCAACTCTA TCGTTGAGAT GGTTATTCTC CCAATAAGAG GCCTAAGGCT	TGGGAGGAAT  ACACCTACCG TGTGGATGGC  ACTCTGTTTA TGAGACAAAT  GGCCAAAGGC CCGGTTTCCG CGGGCTATTC GCCCGATAAG TGTCTTGTGA ACAGAACACT	GGACGGCCT CCTGACCGTA GGACTGGCAT CCAGAACAGG GGTCTTGTCC GCAGCTACTG CGTCGATGAC TAATTCAGGT ATTAAGTCCA TTCTCTTTAT AAGAGAAATA TGTGTGCACA	AACCAGACTT TTGGTCTGAA AGGTGAGCTT TCCACTCGAA TGGGGTTTAT ACCCCAAATA ATCTCTAGAA AAGAGATCTT TCTTATACTA AGAATATGAT ATTGCATTTA
29001 29051 29101 29151 29201	TGAGGTAGTC  GCGTCACCGG CGCAGTGGCC  TTTCCGGACA AAAGGCCTGT  AGAAAACCCT TCTTTTGGGA  GAACAATTCA CTTGTTAAGT  TCGGGGTTGG AGCCCCAACC  ACGCTTCTCT TGCGAAGAGA  TTGTCAGCTT	CCGCTGCACC GGCGACGTGG GACCTCAATA CTGGAGTTAT CTGGAGTATTA ATCCCATAAT ACCCATAAT CGTTGAGAT CCAATAAGAG GCCTAAGGCT CGGATTCCGA TTTAAACGCT	TGGGAGGAAT ACACCTACCG TGTGGATGGC ACTCTGTTTA TGAGACAAAT GGCCAAAGGC CCGGTTTCCG CGGGCTATTC GCCCGATAAG TGTCTTGTGA ACAGAACACT CGCCGCCTGC GCCGGCGACG	GGACGGCCT CCTGACCGTA GGACTGGCAT CCAGAACAGG GGTCTTGTCC GCAGCTACTG CGTCGATGAC TAATTCAGGT ATTAAGTCCA TTCTCTTTAT AAGAGAAATA TGTGTGCACA ACACACGTGT	AACCAGACTT TTGGTCTGAA AGGTGAGCTT TCCACTCGAA TGGGGTTTAT ACCCCAAATA TTCTCTAGAA AAGAGATCTT TCTTATACTA AGAATATGAT TTTGCATTTA AAACGTAAAT TTAGGTACAT
29001 29051 29101 29151 29201 29251 29301	TGAGGTAGTC  GCGTCACCGG CGCAGTGGCC  TTTCCGGACA AAAGGCCTGT  AGAAAACCCT TCTTTTGGGA  GAACAATTCA CTTGTTAAGT  TCGGGGTTGG AGCCCCAACC  ACGCTTCTCT TGCGAAGAGA  TTGTCAGCTT	CCGCTGCACC GGCGACGTGG GACCTCAATA CTGGAGTTAT CTGGAGTATTA ATCCCATAAT AGCAACTCTA TCGTTGAGAT GGTTATTCTC CCAATAAGAG GCCTAAGGCT CTGAGTTCCGA TTTAAACGCT AAATTTGCGA TTACTCACCC	TGGGAGGAAT  ACACCTACCG TGTGGATGGC  ACTCTGTTTA TGAGACAAAT  GGCCAAAGGC CCGGTTTCCG  CGGGCTATTC GCCCGATAAG  TGTCTTGTGA ACAGAACACT  CGCCGCCTGC GCGGCGGACG  GGGGTCGCCA CCCCAGCGGT  TTGCGTCAGC	GGACGGCCT CCTGACCGTA GGACTGGCAT CCAGAACAGG GGTCTTGTCC GCAGCTACTG CGTCGATGAC TAATTCAGGT ATTAAGTCCA TTCTCTTTAT AAGAGAAATA TGTGTGCACA ACACACGTGT CCCAAGATGA GGGTTCTACT CCCACGTACC	AACCAGACTT TTGGTCTGAA AGGTGAGCTT TCCACTCGAA TGGGGTTTAT ACCCCAAATA ATCTCTAGAA AAGAGATCTT TCTTATACTA AGAATATGAT TTTGCATTTA AAACGTAAAT ATTAGGTACAT AATCCATGTA AACCCAAAAGG

Figure 26 AE

29401		GAGAATATTT	TACGTGGTGT	CTTGTACTTT	TCGACGAATA
29451	AGCGGTGTTT	TTGTTTTAAC	GCAAGTATGC CGTTCATACG	ACAAATACGA	TAAACCGTCG
		ATGTCTCATA	TTACAATGTC	AAAAGGTCCC	ATTTTCAGTA
29551	AAAACTTTTA TTTTGAAAAT	TGTATACTTT ACATATGAAA	TCCATTTTAT AGGTAAAATA	GAAATGTGCG CTTTACACGC	ACATTACCAT TGTAATGGTA
29601		TTTGTCATAT	TCAACACCGG	GGGTGTTTTA	ACACACCTTT
29651	TGTGACCGTG	AAAGACGACG	TGACGATACG	ATTAATGTCA	CGAGCGAAAC
29701	CAGACATGGG	ATGAGATATA	TAAATACAAA ATTTATGTTT	TCGTCTGCGT	CGAAATAACT
29751	CCTTTTCTTT	TACGGAATTA	TTACTAAGTT AATGATTCAA	TGTTTCGATT	ACAGTGGTGA
29801 :	TTGACGAAAT	GAGCGACGAA	GCAAAACAAA CGTTTTGTTT	AAGTTTTTCA	ATCGTAATAT
29851	TAATCTTATC	CTAAATTTGG	CCCCGGTCAT GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
29901	GGACTTGTTA	ACTGAGATAC	TGGGATATGC ACCCTATACG	AGGTCGCGAT	GTTGGAACTT
29951	CAGTCCGAAG	GACCTACAGT	GCATCTGACT CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	CCTAAACAAG	GTCAGGTTGA	ACAGCGACCC TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
30051	GTGTTGGTTG	Cecceccec	CTACCGGACT GATGGCCTGA	ATGTAGATGG	TGTTTATGTG
30101	GGGTTCAAAG	ACGGAAACAG	AATAACTGGG TTATTGACCC	TATTGAACCC	GTACACCACC
		GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
		GCGTTTGCGC	GGGCTGGTGG	GTAGATATCA	GGGTAGTAAC
		TTTGTTACTA	CCTTAGGTAT	CTAACCTGCC	TGACTTTGTG
30301	ATGTTCTTTT TACAAGAAAA	CTCTTACAGT GAGAATGTCA	ATGATTAAAT TACTAATTTA	GAGACATGAT CTCTGTACTA	TCCTCGAGTT AGGAGCTCAA

Figure 26 AF

30351	TTTATATTAC AAATATAATG	T CCTTGT ACTGGGAACA			
30401	TGCGGTTTCT ACGCCAAAGA	CACATCGAAG GTGTAGCTTC			
30451		ATTTGTCACC TAAACAGTGG			
30501		TTATCCAGTG AATAGGTCAC			
30551	TCTCAGACAC AGAGTCTGTG	CATCCCCAGT GTAGGGGTCA			
30601		ATTATGAAAT TAATACTTTA			
30651		GTTTTGTTCC CAAAACAAGG			
30701		CTCGTATATG GAGCATATAC			
30751	• • • • • • • • • • • • • • • • • • • •	GAAGCCTGGT CTTCGGACCA			
30801		CTTAGCCCTA GAATCGGGAT			
30851		ATGCCATGAA TACGGTACTT			
30901		CAAGTTGTTG GTTCAACAAC			
30951		TCCCACCCCC AGGGTGGGGG			
31001		GACACCCTAG CTGTGGGATC			
31051		AGAAAGACGC TCTTTCTGCG			
31101	CAAGAGCTCC GTTCTCGAGG	AAGACATGGT TTCTGTACCA			
31151	TTGTCTCGTA AACAGAGCAT	AAGCAGGCCA TTCGTCCGGT			
31201	ACCGCCTTAG TGGCGGAATC	CTACAAGTTG GATGTTCAAC	CCAACCAAGC GGTTGGTTCG	GTCAGAAATT CAGTCTTTAA	GGTGGTCATG CCACCAGTAC
31251	GTGGGAGAAA CACCCTCTTT				AAACCGAAGG TTTGGCTTCC

Figure 26 A6

31301	CTGCATTCAC GACGTAAGTG	TCTTGTC AGTGGAACAG	AAGGACCTGA TTCCTGGACT	GGATCTCTGC CCTAGAGACG	ACCCTT TA TGGGAALAAT
31351	AGACCCTGTG TCTGGGACAC	CGGTCTCAAA GCCAGAGTTT	GATCTTATTC CTAGAATAAG	CCTTTAACTA GGAAATTGAT	ATAAAAAAA TATTTTTTTT
31401	TATTATTTCG	ATCACTTACT TAGTGAATGA	ATTTTAGTCA	ATCGTTTAAA	GACAGGTCAA
31451	TATTCAGCAG ATAAGTCGTC	CACCTCCTTG GTGGAGGAAC	CCCTCCTCCC	AGCTCTGGTA TCGAGACCAT	TTGCAGCTTC AACGTCGAAG
31501	CTCCTGGCTG GAGGACCGAC	CAAACTTTCT GTTTGAAAGA	CCACAATCTA GGTGTTAGAT	AATGGAATGT TTACCTTACA	CAGTTTCCTC GTCAAAGGAG
31551	GACAAGGACA	CCATCCGCAC GGTAGGCGTG	GGTGATAGAA	GTACAACAAC	GTCTACTTCG
31601	CGCGTTCTGG	GTCTGAAGAT CAGACTTCTA	TGGAAGTTGG	GGCACATAGG	TATACTGTGC
31651	CTTTGGCCAG	CTCCAACTGT GAGGTTGACA	CGGAAAAGAA	TGAGGAGGGA	AACATAGGGG
.31701	GTTACCCAAA	CAAGAGAGTC GTTCTCTCAG	GGGGACCCCA	TGAGAGAAAC	GCGGATAGGC
31751	TTGGAGATCA	TACCTCCAAT ATGGAGGTTA	CCGTACGAAC	GCGAGTTTTA	CCCGTTGCCG
31801	GAGAGAGACC	ACGAGGCCGG TGCTCCGGCC	GTTGGAATGG	AGGGTTTTAC	ATTGGTGACA
31851	CTCGGGTGGA	CTCAAAAAAA GAGTTTTTTT	GGTTCAGTTT	GTATTTGGAC	CTTTATAGAC
31901	GTGGGGAGTG	AGTTACCTCA TCAATGGAGT	CTTCGGGATT	GACACCGACG	GCGGCGTGGA
31951	GATTACCAGC	CGGGCAACAC GCCCGTTGTG	TGAGTGGTAC	GTTAGTGTCC	GGGGCGATTG
32001	GCACGTGCTG	AGGTTTGAAT	CGTAACGGTG	GGTTCCTGGG	CTCACAGTGT GAGTGTCACA
	GTCTTCCTTT	CGATCGGGAC	GTTTGTAGTC	CGGGGGAGTG	CACCACCGAT GTGGTGGCTA
	TCGTCATGGG	AATGATAGTG	ACGGAGTGGG	GGAGATTGAT	CTGCCACTGG GACGGTGACC
	ATCGAACCCG	TAACTGAACT	TTCTCGGGTA	AATATGTGTT	AATGGAAAAC TTACCTTTTG
32201	TAGGACTAAA ATCCTGATTT	GTACGGGGCT CATGCCCCGA	CCTTTGCATG GGAAACGTAC	TAACAGACGA ATTGTCTGCT	CCTAAACACT GGATTTGTGA

Figure 26 AH

32251		AGGTGTGACT TCCACACTGA		
32301		 TGGGTTTTGA ACCCAAAACT		
32351		AGGATTGATT TCCTAACTAA		
32401		TGATGCTCAA ACTACGAGTT		
32451	AGGACAGGGC TCCTGTCCCG	TAAACTCAGC ATTTGAGTCG		
32501		 TTTACAGCTT AAATGTCGAA		CAAAAAGCTT GTTTTTCGAA
32551		 CAAGGGGTTG GTTCCCCAAC		
32601		 GGCTTGAATT CCGAACTTAA		
32651		 AAAATTGGCC TTTTAACCGG		
32701		ACTAGGAACT TGATCCTTGA		
32751		'ACAAAAATAA TGTTTTTATT	_	
32801		AACTGTAGAC TTGACATCTG		
32851		AAAATGTGGC TTTTACACCG		
32901		 GCAGTTTGGC CGTCAAACCG		
32951		 AGATTTGACG TCTAAACTGC		
33001	AATTCCTTCC TTAAGGAAGG	 ATATTGGAAC TATAACCTTG		
33051	TGAAGGCACA ACTTCCGTGT	ACGCTGTTGG TGCGACAACC		
33101	CTTATCCAAA GAATAGGTTT	AAAACTGCCA TTTTGACGGT		
33151	GTTTACTTAA CAAATGAATT	AACTAAACCT TTGATTTGGA		

Figure 26 AI

33201	AAACGGTACA TTTGCCATGT	GAAACAG GACTTTGTC	GAGACACAAC CTCTGTGTTG	TCCAAGTGČA AGGTTCACGT	TACTCT TT ATGAGACA
33251				ACATTAATGA TGTAATTACT	
33301				CAAGAATAAA GTTCTTATTT	
33351				TGCAGAAAAT ACGTCTTTTA	
33401	TTTTCATTCA AAAAGTAAGT			CATAGCTTAT GTATCGAATA	
33451				ATTCAACCTG TAAGTTGGAC	
33501				CCCGGCTGGC GGGCCGACCG	
33551				GGTGTTATAT CCACAATATA	
33601				ATTAATAAAC TAATTATTTG	
33651				GCTGAGCCAC CGACTCGGTG	
33701	CCAACTTGCG GGTTGAACGC	GTTGCTTAAC CAACGAATTG	GGGCGGCGAA CCCGCCGCTT	GGAGAAGTCC CCTCTTCAGG	ACGCCTACAT TGCGGATGTA
33751				AGGGCGGTGG TCCCGCCACC	
33801	GCGCGCGAAT CGCGCGCTTA	AAACTGCTGC TTTGACGACG	CGCCGCCGCT	CCGTCCTGCA GGCAGGACGT	GGAATACAAC CCTTATGTTG
33851				ACCGCCCGCA TGGCGGGGGT	
33901	CCTTGTCCTC GGAACAGGAG	CGGGCACAGC GCCCGTGTCG	AGCGCACCCT TCGCGTGGGA	GATCTCACTT CTAGAGTGAA	AAATCAGCAC TTTAGTCGTG
33951	AGTAACTGCA TCATTGACGT	GCACAGCACC CGTGTCGTGG	ACARTATTGT TGTTATAACA	TCAAAATCCC AGTTTTAGGG	ACAGTGCAAG TGTCACGTTC
34001	GCGCTGTATC CGCGACATAG	CAAAGCTCAT GTTTCGAGTA	GGCGGGGACC CCGCCCCTGG	ACAGAACCCA TGTCTTGGGT	CGTGGCCATC GCACCGGTAG
34051	ATACCACAAG TATGGTGTTC	CGCAGGTAGA GCGTCCATCT	TTAAGTGGCG AATTCACCGC	ACCCCTCATA TGGGGAGTAT	AACACGCTGG TTGTGCGACC
34101	ACATAAACAT TGTATTTGTA	TACCTCTTTT ATGGAGAAAA	GGCATGTTGT CCGTACAACA	AATTCACCAC TTAAGTGGTG	CTCCCGGTAC GAGGGCCATG

Figure 26 AJ

34151	CATATAAACC GTATATTTGG	T CATTAAA ACTAATTT	CATGGCGCCA GTACCGCGGT	TCCACCACCAT AGGTGGTGGT	TCCTAA TA AGGATT ST
34201				CTGCAGGGAA GACGTCCCTT	
34251				AACCATGGAT TTGGTACCTA	
34301				CACACGTGCA GTGTGCACGT	
34351				CATATCCCAG GTATAGGGTC	
34401				AGGGAAGACC TCCCTTCTGG	
34451				TCGGGCAGCA AGCCCGTCGT	
34501				AAAAGGAGGT TTTTCCTCCA	
34551				ATCGTGTTGG TAGCACAACC	
34601				TTTCCTGAAG AAAGGACTTC	
34651				GGTCTCGCCG CCAGAGCGGC	
34701				CTCAAAGCAT GAGTTTCGTA	
34751				ATGCGCCGCT TACGCGGCGA	
34801	CATCCACCAC GTAGGTGGTG			GCCAACCTAC CGGTTGGATG	
34851				GCTGGAAGAA CGACCTTCTT	
34901	TTTTTTATTC AAAAAATAAG			CAAAATGAAG GTTTTACTTC	
34951	TGAACGCGCT ACTTGCGCGA			AACTCTACAG TTGAGATGTC	
35001	GATAATGGCA CTATTACCGT			GGCTTCCAAA CCGAAGGTTT	
35051	CCCTCACGTC GGGAGTGCAG			ACCCTTCAGG TGGGAAGTCC	

Figure 26 AK

35101	TCTATAAACA AGATATTTGT	TAGCACC AAGGTCGTGG	TTCAACCATG AAGTTGGTAC	CCCAAATAAT GGGTTTATTA	TCTCAT G AGAGTAGAGC
35151				CCGAATATTA GGCTTATAAT	
35201	AACATTTTTA	GACGAGGTCT	CGCGGGAGGT	CCTTCAGCCT GGAAGTCGGA	GTTCGTCGCT
35251				AGACCTGTAT TCTGGACATA	
35301	AGCGGAACAT TCGCCTTGTA	TAACAAAAAT ATTGTTTTTA	ACCGCGATCC TGGCGCTAGG	CGTAGGTCCC GCATCCAGGG	TTCGCAGGGC AAGCGTCCCG
35351	GTCGACTTGT	ATTAGCACGT	CCAGACGTGC	GACCAGCGCG CTGGTCGCGC	CGGTGAAGGG
35401	GCGGTCCTTG	GTACTGTTTT	CTTGGGTGTG	TGATTATGAC ACTAATACTG	TGCGTATGAG
35451	CCTCGATACG	ATTGGTCGCA	TCGGGGCTAC	TAAGCTTGTT ATTCGAACAA	CGTACCCGCC
35501	GCTATATTTT	ACGTTCCACG	ACGAGTTTTT	ATCAGGCAAA TAGTCCGTTT	CGGAGCGCGT
35551	TTTTTTTTTC	GTGTAGCATC	AGTACGAGTA	GCAGATAAAG CGTCTATTTC	CGTCCATTCG
35601	AGGCCTTGGT	GGTGTCTTTT	TCTGTGGTAA	TTTCTCTCAA AAAGAGAGTT	TGTACAGACG
35651	CCCAAAGACG	TATTTGTGTT	TTATTTTATT	CAAAAAAACA GTTTTTTGT	AAATTTGTAA
35701	TCTTCGGACA	GAATGTTGTC	CTTTTTGTTG	GGAATATTCG	
35751		CGGCCGCACT	GGCATTTTTT	TGACCAGTGG	CACTAATTTT
35801	TCGTGGTGGC	TGTCGAGGAG	CCAGTACAGG	GGAGTCATAA CCTCAGTATT	ACATTCTGAG
		AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
•		CCCTTATGTA	TEGGCGTCCG	CATCTCTGTT	GTAATGTCGG
		CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
36001	TGAAAAACCC ACTTTTTGGG	TCCTGCCTAG AGGACGGATC	GCAAAATAGC CGTTTTATCG	ACCCTCCCGC TGGGAGGGCG	TCCAGAACAA AGGTCTTGTT

Figure 26 AL

36051	CATACAGCGC GTATGTCGCG		GCAGCCATAA CGTCGGTATT		
36101			ACACCACTCG TGTGGTGAGC		
36151			CAAGTGCAGA GTTCACGTCT		
36201			GTCCACAAAA CAGGTGTTTT		
					•
36251			AAAGCCAAAA TTTCGGTTTT		
36301	CGTCACTTCC GCAGTGAAGG	GTTTTCCCAC CAAAAGGGTG	GTTACGTCAC CAATGCAGTG	TTCCCATTIT AAGGGTAAAA	AAGAAAACTA TTCTTTTGAT
36351	CAATTCCCAA	CACATACAAG	TTACTCCGCC	CTAAAACCTA	CGTCACCCGC
50002			AATGAGGCGG		
36401			CACGTCACAA GTGCAGTGTT		
					PacI
36451	<u> </u>	<b>הממממרטייה</b>	AAGGTATATT	ATTGATGATG	TTAATTAAGA
30431			TTCCATATAA		
36501			GCTGGATGGC		
	TAAGCCTAGA	CGCTGCGCTC	CGACCTACCG	GAAGGGGTAA	TACTAAGAAG-
36551	TCGCTTCCGG	CGGCATCGGG	ATGCCCGCGT	TGCAGGCCAŢ	GCTGTCCAGG
			TACGGGCGCA		
36601			GGGACAGCTT		
	GTCCATCTAC	TGCTGGTAGT	CCCTGTCGAA	GTTCCGGTCG	TTTTCCGGTC
36651			TGCTGGCGTT		
•	CTTGGCATTT	TTCCGGCGCA	ACGACCGCAA	AAAGGTATCC	GAGGCGGGGG
36701	00000000000				
			CGACGCTCAA GCTGCGAGTT		
36751	GACTGCTCGT	AGTGTTTTTA	GCTGCGAGTT	CAGTCTCCAC	CGCTTTGGGC
36751	GACTGCTCGT ACAGGACTAT	AGTGTTTTTA  AAAGATACCA	GCTGCGAGTT GGCGTTTCCC	CAGTCTCCAC	CGCTTTGGGC
	GACTGCTCGT ACAGGACTAT TGTCCTGATA CTCTCCTGTT	AGTGTTTTA  AAAGATACCA TTTCTATGGT  CCGACCCTGC	GCTGCGAGTT GGCGTTTCCC CCGCAAAGGG CGCTTACCGG	CAGTCTCCAC CCTGGAAGCT GGACCTTCGA ATACCTGTCC	CCCTTTGGGC CCCTCGTGCG GGGAGCACGC GCCTTTCTCC
	GACTGCTCGT ACAGGACTAT TGTCCTGATA	AGTGTTTTA  AAAGATACCA TTTCTATGGT  CCGACCCTGC	GCTGCGAGTT GGCGTTTCCC CCGCAAAGGG CGCTTACCGG	CAGTCTCCAC CCTGGAAGCT GGACCTTCGA ATACCTGTCC	CCCTTTGGGC CCCTCGTGCG GGGAGCACGC GCCTTTCTCC
36801	GACTGCTCGT ACAGGACTAT TGTCCTGATA CTCTCCTGTT GAGAGGACAA CTTCGGGAAG	AGTGTTTTTA  AAAGATACCA TTTCTATGGT  CCGACCCTGC GGCTGGGACG  CGTGGCGCTT	GCTGCGAGTT GGCGTTTCCC CCGCAAAGGG CGCTTACCGG GCGAATGGCC TCTCATAGCT	CAGTCTCCAC CCTGGAAGCT GGACCTTCGA ATACCTGTCC TATGGACAGG CACGCTGTAG	CCCTTTGGGC CCCTCGTGCG GGGAGCACGC GCCTTTCTCC CGGAAAGAGG GTATCTCAGT
36801	GACTGCTCGT ACAGGACTAT TGTCCTGATA CTCTCCTGTT GAGAGGACAA CTTCGGGAAG	AGTGTTTTTA  AAAGATACCA TTTCTATGGT  CCGACCCTGC GGCTGGGACG  CGTGGCGCTT	GCTGCGAGTT GGCGTTTCCC CCGCAAAGGG CGCTTACCGG GCGAATGGCC TCTCATAGCT	CAGTCTCCAC CCTGGAAGCT GGACCTTCGA ATACCTGTCC TATGGACAGG CACGCTGTAG	CCCTTTGGGC CCCTCGTGCG GGGAGCACGC GCCTTTCTCC CGGAAAGAGG
36801 36851	GACTGCTCGT ACAGGACTAT TGTCCTGATA CTCTCCTGTT GAGAGGACAA CTTCGGGAAG	AGTGTTTTTA  AAAGATACCA TTTCTATGGT  CCGACCCTGC GGCTGGGACG  CGTGGCGCTT GCACCGCGAA  TCGTTCGCTC	GCTGCGAGTT GGCGTTTCCC CCGCAAAGGG CGCTTACCGG GCGAATGGCC TCTCATAGCT AGAGTATCGA CAAGCTGGGC	CAGTCTCCAC CCTGGAAGCT GGACCTTCGA ATACCTGTCC TATGGACAGG CACGCTGTAG GTGCGACATC TGTGTGCACGC	CCCTTTGGGC CCCTTGCTGCG GGGAGCACGC GCCTTTCTCC CGGAAAGAGG GTATCTCAGT CATAGAGTCA AACCCCCCGT

Figure 26 AM

36951	AGTCGGGCTG	GCGACGCGGA	TATCCGGTAA ATAGGCCATT	GATAGCAGAA	CTCAGG
37001	CGGTAAGACA	CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT
	GCCATTCTGT	GCTGAATAGC	GGTGACCGTC	GTCGGTGACC	ATTGTCCTAA
37051	AGCAGAGCGA	GGTATGTAGG	CGGTGCTACA	GAGTTCTTGA	AGTGGTGGCC
	TCGTCTCGCT	CCATACATCC	GCCACGATGT	CTCAAGAACT	TCACCACCGG
37101	TAACTACGGC	TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA
	ATTGATGCCG	ATGTGATCTT	CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151	AGCCAGTTAC	CTTCGGAAAA	AGAGTTGGTA	GCTCTTGATC	CGGCAAACAA
	TCGGTCAATG	GAAGCCTTTT	TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT
37201	ACCACCGCTG	GTAGCGGTGG	TTTTTTTGTT	TGCAAGCAGC	AGATTACGCG
	TGGTGGCGAC	CATCGCCACC	AAAAAAACAA	ACGTTCGTCG	TCTAATGCGC
37251	CAGAAAAAA	GGATCTCAAG	AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG
	GTCTTTTTT	CCTAGAGTTC	TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC
37301	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTTGGT	CATGAGATTA
	TGCGAGTCAC	CTTGCTTTTG	AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351	'TCAAAAAGGA	TCTTCACCTA	GATCCTTTTA	AATCAATCTA	AAGTATATAT
	AGTTTTTCCT	AGAAGTGGAT	CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT
	CTCATTTGAA	CCAGACTGTC	AATGGTTACG	AATTAGTCAC	TCCGTGGATA
37451	CTCAGCGATC	TGTCTATTTC	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG
	GAGTCGCTAG	ACAGATAAAG	CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA
	ACATCTATTG	ATGCTATGCC	CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551	TACTATGGCG	CTCTGGGTGC	CTCACCGGCT GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
37601	GGTCGGTCGG	CCTTCCCGGC	AGCGCAGAAG TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	GGAGGTAGGT	CAGATAATTA	ACAACGGCCC	TTCGATCTCA	
37701	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	GCATCGTGGT CGTAGCACCA
37751	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT
	CAGTGCGAGC	AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	AGGGTTGCTA
	GTTCCGCTCA	ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	GGTTAGCTCC CCAATCGAGG
37851	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT
	AAGCCAGGAG	GCTAGCAACA	GTCTTCATTC	AACCGGCGTC	ACAATAGTGA

Figure 26 AN

37901	CATGGTTATG GTACCAATAC		ATAATTCTCT TATTAAGAGA		
37951	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG
	CTACGAAAAG	ACACTGACCA	CTCATGAGTT	GGTTCAGTAA	GACTCTTATC
38001	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAACAC	GGGATAATAC
			GAGAACGGGC		
38051	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT
	GCGCGGTGTA	TCGTCTTGAA	ATTTTCACGA	GTAGTAACCT	TTTGCAAGAA
38101	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG
•	GCCCCGCTTT	TGAGAGTTCC	TAGAATGGCG	ACAACTCTAG	GTCAAGCTAC
38151	TAACCCACTC				
	ATTGGGTGAG	CACGTGGGTT	GACTAGAAGT	CGTAGAAAAT	GAAAGTGGTC
38201			CAGGAAGGCA		
	GCAAAGACCC	ACTCGTTTTT	GTCCTTCCGT	TTTACGGCGT	TTTTTCCCTT
38251			TGAATACTCA		
	ATTCCCGCTG	TGCCTTTACA	ACTTATGAGT	atgagaagga	AAAAGTTATA
38301			TTATTGTCTC		
	ATAACTTCGT	AAATAGTCCC	AATAACAGAG	TACTCGCCTA	TGTATAAACT
38351	ATGTATTTAG				
	TACATAAATC	TTTTTATTTG	TTTATCCCCA	AGGCGCGTGT	AAAGGGGCTT
38401			GAAACCATTA		
	TTCACGGTGG	ACTGCAGATT	CTTTGGTAAT	AATAGTACTG	TAATTGGATA
38451	AAAAATAGGC				
	TTTTTATCCG	CATAGTGCTC	CGGGAAAGCA	GAAGTTCTTA	ACCTAGGCTT
•		PacI			
		~~~~~			
38501	TTCTTAATTT				
	AAGAATTAAA	GAATTAATT	(SEQ ID NO:	33)	

Figure 26 AO

## MRKAd5nef MER1063 (MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51	CCCCTCCACT	TTGTGACGTG	ececeeece	TGGGAACGGG	GCGGGTGACG
JI	GGGGIGGAG1	AACACTGCAC	CCCCCCCCC	ACCCTTGCCC	CGCCCACTGC
	CCCCACCTCA	WHENCIGENE	COCOCCCOC	ACCC110CCC	000000.0100
			~> mammaa> >	CDCDCCC3	3C3C8DCD33
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GIGIGGCGGA	MCMCW1G1WW
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201		TTTTCGCGCG			
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
	00				
301	አርጥር እ አ አጥር ጥ	CAACAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
301	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCCCCCATT	ATAAACAGAT
	ICACITIAGA	CI IMITICAL.			
351	0000000000	GACTTTGACC	COMMANDER	AGACTCGCCC	<b>Σ</b> ርርጥር ተዋጥጥ
221	0000000000	CTGAAACTGG	CARAMOCRO	MCTCACCCC	TCCACAAAA
	CCCGCGCGCCC	CIGNAMCIGG	CAMAIGCACC	101000000	
		TTCCGCGTTC	CCCCCCAAAC	MACCCC TOTAL	אַריימיימיימיימיימיימיימיימיימיימיימיימיימ
401	CTCAGGTGTT	TTCCGCGTTC	CGGGICAAAG	7700007777	WY 2
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
					m> mom> o> mm
451	GCGGCCGCGA	TCCATTGCAT	ACGITGIATC	CATATCATAA	TAIGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
012	ACCTCAAGGC:	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
657	CCCMMCCMCC	GGGCGGGTAA	CUCCACUTATI	TACTGCATAC	AAGGGTATCA
	6661166166	66666661131	01001011111	21.02.00.12.10	
	******	GGGACTTTCC	annica conca	ATTECCTOCAC	<b>ТЕПТЕТ В ТЕПТЕТ</b>
701	AACGCCAATA	GGGACTITCC	TA A CONCOLUCA	TACCCACCTC	ATAAATGCCA
	TIGCGGTTAT	CCCTGMMAGG	TWWC TOCKOL	INCCUNCTIC	
		CTTGGCAGTA	0 x m0 x x 0 m0 m	3mm3m200	AACTACCCC
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGIGT	MICHIAIGCC	WHO INCOCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
					> m00000> 000
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

851	CATGACCTTA GTACTGGAAT	TEACTTTC ACCTGAAAG	CTACTTGGCA GATGAACCGT	GTACATCTAC CATGTAGATG	GTATTA TA CATAATCAGT
. 901			CGGTTTTGGC GCCAAAACCG		
951	TAGCGGTTTG ATCGCCAAAC		ATTTCCAAGT TAAAGGTTCA		
1001			AAAATCAACG TTTTAGITGC		
1051			CAAATGGGCG GTTTACCCGC		
1101			TTTAGTGAAC AAATCACTTG		
1151			TCCATAGAAG AGGTATCTTC		
1201			ATTGGAACGC TAACCTTGCG		
1251			GCAAGTGGTC CGTTCACCAG		
1301			ATGAGGAGGG TACTCCTCCC		
1351			CGCAGTGGGC GCGTCACCCG		
1401			TCACCTCCTC AGTGGAGGAG		
1451			GCCCAGGAGG CGGGTCCTCC		
1501	*		GAGGCCCATG CTCCGGGTAC		
1551			AGAAGGGCGG TCTTCCCGCC		
1601	CCCAGAAGAG GGGTCTTCTC		CTGGACCTGT GACCTGGACA		
1651	TACTTCCCCG ATGAAGGGGC		CTACACCCCC GATGTGGGGG		
1701	CCTGACCTTC GGACTGGAAG		TCAAGCTGGT AGTTCGACCA		
1751	TGGAGGAGGC ACCTCCTCCG				

Figure 27B

1801	CAGCACGGCA GTCGTGCCGT	TAGGACCC AGETCCTGGG	CGAGAAGGAG GCTCTTCCTC	CTCCTCGAGT CACGACCTCA	GGAGGT A CCTCCAAGCT
1851	CTCCAAGCTG GAGGTTCGAC			GGAGCTGCAC CCTCGACGTG	
1901				CTGTGCCTTC GACACGGAAG	
1951				TCCTTGACCC AGGAACTGGG	
2001				GGAAATTGCA CCTTTAACGT	
2051				GGGTGGGGCA CCCACCCGT	
2101				GCTGGGGATG CGACCCCTAC	
2151	TATGGCCGAT ATACCGGCTA			TGTGGGCGTG ACACCCGCAC	
2201				GTAGTTTTGT CATCAAAACA	
2251				CGTTTGATGG GCAAACTACC	
2301				TGGGCCGGG	
2351				CGTCCTGCCC	
2401				CGCCGTTGGA GCGGCAACCT	
2451				GCCCGCGGA CGGCGCCCT	
2501	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	TGCAGCTTCC ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA GGGCGCTACT	CAAGTTGACG GTTCAACTGC	GCTCTTTTGG CGAGAAAACC	CACAATTGGA GTGTTAACCT	TTCTTTGACC AAGAAACTGG
2601					GCCAGCAGGT CGGTCGTCCA
2651	TTCTGCCCTG AAGACGGGAC			TGCGGTTTAA ACGCCAAATT	
2701	AAAAACCAGA TTTTTGGTCT				TTGCTGTCTT AACGACAGAA

Figure 27C

2751	TATTTAGGGG	TITTGCGCGC	GCGGTAGGCC	CGGGACCAGC	GGTCTCGGTC
		AAAACGCGCG			
2801	GTTGAGGGTC CAACTCCCAG	CTGTGTATTT GACACATAAA			
2851		CATGGGCATA GTACCCGTAT			
2901	TGCAGAGCTT ACGTCTCGAA	CATGCTGCGG GTACGACGCC			
2951		GCGTGGTGCC CGCACCACGG			
3001		GCCCTTGGTG CGGGAACCAC			
3051		GTGGGGATAT CACCCCTATA			
3101		CCAGCCATAT GGTCGGTATA			
3151	CCAGCACAGT GGTCGTGTCA	GTATCCGGTG CATAGGCCAC	CACTTGGGAA GTGAACCCTT	ATTTGTCATG TAAACAGTAC	TAGCTTAGAA ATCGAATCTT
3201		GGAAGAACTT CCTTCTTGAA			
3251		TCCATAATGA AGGTATTACT			
3301		TCTGGGATCA AGACCCTAGT			
3351		CCATTTTTAC GGTAAAAATG			
3401		CCATCCGGCC GGTAGGCCGG			
3451		TTTGAGTTCA AAACTCAAGT			
3501	ATGAAGAAAA TACTTCTTTT				AAGAAAGCAG TTCTTTCGTC
3551	GTTCCTGAGC CAAGGACTCG				TAAATCACAC ATTTAGTGTG
3601	CTATTACCGG GATAATGGCC				GCCGTCATCC CGGCAGTAGG
3651	CTGAGCAGGG GACTCGTCCC				GCATGTTTTC CGTACAAAAG

Figure 270

3701	CCTGACCAAA	ECCAGAA	GCGCTCGCC	GCCCAGCGAT	AGCAGT TT
	GGACTGGTTT	AGGCGGTCTT	CCGCGAGCGG	CGGGTCGCTA	TCGTCAAGAA
3751	GCAAGGAAGC	AAAGTTTTTC	AACGGTTTGA	GACCGTCCGC	CGTAGGCATG
	CGTTCCTTCG	TTTCAAAAAG	TTGCCAAACT	CTGGCAGGCG	GCATCCGTAC
3801	CTTTTGAGCG GAAAACTCGC			CGGTCCCACA GCCAGGGTGT	
3851				TCCTCGTTTC AGGAGCAAAG	
3901	GCGGCTTTCG	CTGTACGGCA	GTAGTCGGTG	CTCGTCCAGA	CGGGCCAGGG
	CGCCGAAAGC	GACATGCCGT	CATCAGCCAC	GAGCAGGTCT	GCCCGGTCCC
3951	TCATGTCTTT	CCACGGGCGC	AGGGTCCTCG	TCAGCGTAGT	CTGGGTCACG
	AGTACAGAAA	GCTGCCCGCG	TCCCAGGAGC	AGTCGCATCA	GACCCAGTGC
4001	GTGAAGGGGT	GCGCTCCGGG	CTGCGCGCTG	GCCAGGGTGC	GCTTGAGGCT
	CACTTCCCCA	CGCGAGGCCC	GACGCGCGAC	CGGTCCCACG	CGAACTCCGA
4051	GGTCCTGCTG	GTGCTGAAGC	GCTGCCGGTC	TTCGCCCTGC	GCGTCGGCCA
	CCAGGACGAC	CACGACTTCG	CGACGGCCAG	AAGCGGGACG	CGCAGCCGGT
4101	GGTAGCATTT CCATCGTAAA	GACCATGGTG CTGGTACCAC	TCATAGTCCA AGTATCAGGT	GCCCCTCCGC	GGCGTGGCCC CCGCACCGGG
4151 ,	TTGGCGCGCA	GCTTGCCCTT	GGAGGAGGCG	CCGCACGAGG	GGCAGTGCAG
	AACCGCGCGT	CGAACGGGAA	CCTCCTCCGC	GGCGTGCTCC	CCGTCACGTC
4201	ACTTTTGAGG	GCGTAGAGCT	TGGGCGCGAG	AAATACCGAT	TCCGGGGAGT
	TGAAAACTCC	CGCATCTCGA	ACCCGCGCTC	TTTATGGCTA	AGGCCCCTCA
4251	AGGCATCCGC	GCCGCAGGCC	CCGCAGACGG	TCTCGCATTC	CACGAGCCAG
	TCCGTAGGCG	CGGCGTCCGG	GGCGTCTGCC	AGAGCGTAAG	GTGCTCGGTC
4301	GTGAGCTCTG	GCCGTTCGGG	GTCAAAAACC	AGGTTTCCCC	CATGCTTTTT
	CACTCGAGAC	CGGCAAGCCC	CAGTTTTTGG	TCCAAAGGGG	GTACGAAAAA
4351	GATGCGTTTC	TTACCTCTGG	TTTCCATGAG	CCGGTGTCCA	CGCTCGGTGA
	CTACGCAAAG	AATGGAGACC	AAAGGTACTC	GGCCACAGGT	GCGAGCCACT
4401	CGAAAAGGCT	GTCCGTGTCC	CCGTATACAG	ACTTGAGAGG	CCTGTCCTCG
	GCTTTTCCGA	CAGGCACAGG	GGCATATGTC	TGAACTCTCC	GGACAGGAGC
4451	AGCGGTGTTC	CGCGGTCCTC	CTCGTATAGA	AACTCGGACC	ACTCTGAGAC
	TCGCCACAAG	GCGCCAGGAG	GAGCATATCT	TTGAGCCTGG	TGAGACTCTG
4501	AAAGGCTCGC	GTCCAGGCCA	GCACGAAGGA	GGCTAAGTGG	GAGGGGTAGC
	TTTCCGAGCG	CAGGTCCGGT	CGTGCTTCCT	CCGATTCACC	CTCCCCATCG
4551	GGTCGTTGTC	CACTAGGGGG	TCCACTCGCT	CCAGGGTGTG	AAGACACATG
	CCAGCAACAG	GTGATCCCCC	AGGTGAGCGA	GGTCCCACAC	TTCTGTGTAC
4601	TCGCCCTCTT	CGCCATCAAG	GAAGGTGATT	GGTTTGTAGG	TGTAGGCCAC
	AGCGGGAGAA	GCCGTAGTTC	CTTCCACTAA	CCAAACATCC	ACATCCGGTG

Figure 27E

4651	GTGACCGGGT CACTGGCCCA			GGGGCC PT CCCCGCGCAA
4701			CGAGGGCCAG GCTCCCGGTC	CTGTTGGGGT GACAACCCCA
4751	GAGTACTCCC CTCATGAGGG		TCTGCGCTAA AGACGCGATT	
4801		 	CTGGCCCGCG GACCGGGCGC	-
4851			AGACAATCTT TCTGTTAGAA	
4901			TTGGACAGCA AACCTGTCGT	
4951			GGCGCGCTCC CCGCGCGAGG	
5001	TGTTTAGCTG ACAAATCGAC	 	ACCGCCATTC TGGCGGTAAG	
5051		 	CGCCAACCGC GCGGTTGGCG	
5101		 	TCCGCGTAGG AGGCGCATCC	
5151			AGAATGGCGG TCTTACCGCC	
5201		 	ACGGTAAAGA TGCCATTTCT	
5251			TCCTTGCAAG AGGAACGTTC	
5301			CGTATGGGTT GCATACCCAA	CTCACCCCCT
5351				CGCAAATGTC GCGTTTACAG
5401	GTAAACGTAG CATTTGCATC			GGGTAGCATC CCCATCGTAG
5451	TTCCACCGCG AAGGTGGCGC			CTCCCAGGGA CACCCTCCCT
5501	GCGAGGAGGT CGCTCCTCCA			CTGCTCGGAA GACGAGCCTT
5551				GTTGGACGCT CAACCTGCGA

Figure 27F

5601	GGAAGACGTT CCTTCTGCAA	CTTCGACCGC	TCTGTGAGAC AGACACTCTG	CTACCGCGTC GATGGCGCAG	ACGCAC G TGCGTGCTTC
5651	GAGGCGTAGG	AGTCGCGCAG	CTTGTTGACC	AGCTCGGCGG	TGACCTGCAC
	CTCCGCATCC	TCAGCGCGTC	GAACAACTGG	TCGAGCCGCC	ACTGGACGTG
5701	GTCTAGGGCG	CAGTAGTCCA	GGGTTTCCTT	GATGATGTCA	TACTTATCCT
	CAGATCCCGC	GTCATCAGGT	CCCAAAGGAA	CTACTACAGT	ATGAATAGGA
5751				GGACAAACTC CCTGTTTGAG	
5801				GCCTCCGAAC CGGAGGCTTG	
5851	TAGCATGTAG	AACTGGTTGA	CGGCCTGGTA	GGCGCAGCAT	CCCTTTTCTA
	ATCGTACATC	TTGACCAACT	GCCGGACCAT	CCGCGTCGTA	GGGAAAAGAT
5901	CGGGTAGCGC	GTATGCCTGC	GCGGCCTTCC	GGAGCGAGGT	GTGGGTGAGC
	GCCCATCGCG	CATACGGACG	CGCCGGAAGG	CCTCGCTCCA	CACCCACTCG
5951	CSTTTCCACA	GGGACTGGTA	CTGAAACTCC		ACTTCAGTCA
6001	GTCGTCGCAT	CCGCCCTGCT	CCCAGAGCAA	AAAGTCCGTG	CGCTTTTTGG
	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	TTTCAGGCAC	GCGAAAAACC
6051				CGTTGAAGAG GCAACTTCTC	
6101	GCGCGAGGCA	TAAAGTTGCG	TGTGATGCGG	AAGGGTCCCG	GCACCTCGGA
	CGCGCTCCGT	ATTTCAACGC	ACACTACGCC	TTCCCAGGGC	CGTGGAGCCT
6151	ACGGTTGTTA	ATTACCTGGG	CGGCGAGCAC	GATCTCGTCA	AAGCCGTTGA
	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	CTAGAGCAGT	TTCGGCAACT
6201	TGTTGTGGCC	CACAATGTAA	AGTTCCAAGA	AGCGCGGGAT	GCCCTTGATG
	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	TCGCGCCCTA	CGGGAACTAC
6251				AGCTCTTCAG TCGAGAAGTC	
6301	CCCGTGCTCT	GAAAGGGCCC	AGTCTGCAAG	ATGAGGGTTG	GAAGCGACGA
	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	CTTCGCTGCT
6351	ATGAGCTCCA	CAGGTCACGG	GCCATTAGCA	TTTGCAGGTG	GTCGCGAAAG
	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
6401	GTCCTAAACT	GGCGACCTAT	GGCCATTTTT	TCTGGGGTGA	TGCAGTAGAA
	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
6451	GGTAAGCGGG	TCTTGTTCCC	AGCGGTCCCA	TCCAAGGTTC	GCGGCTAGGT
	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC	AGTCACTAGA	GGCTCATCTC	CGCCGAACTT	CATGACCAGC
	GAGCGCGCCG	TCAGTGATCT	CCGAGTAGAG	GCGGCTTGAA	GTACTGGTCG

Figure 27G

6551 [°]	ATGAAGGGCA	CTCTT	CCCAAAGGCC	CCCATCCAAG	TATAGG
	TACTTCCCGT	GCTCGACGAA	GGGTTTCCGG	GGGTAGGTTC	ATATCCAGAG
6601				GCGAGGATGC	
		•		CGCTCCTACG	
6651				AGGAGTGGCT TCCTCACCGA	
	CCTTCTTGAC	CIAGAGGGCG	GIGGITANCC	ICCICACCOA	IMAL INCACC
6701				CACTCGTGCT	
6751	AAAACGTGCG	CAGTACTGGC	AGCGGTGCAC	GGGCTGTACA CCCGACATGT	TCCTGCACGA
	TTTTGCACGC	GTCATGACCG	TOGOCACGIG	CCCGACATGT	AGGACGTGCT
6801	GGTTGACCTG	ACGACCGCGC	ACAAGGAAGC	AGAGTGGGAA	TTTGAGCCCC
				TCTCACCCTT	
6851	TCGCCTGGCG	CGTTTCGCTC	GTGGTCTTCT	ACTTCGGCTG	CTTGTCCTTG
	AGCGGACCGC	CCAAACCGAC	CACCAGAAGA	TGAAGCCGAC	GAACAGGAAC
6901	ACCGTCTGGC	TGCTCGAGGG	GAGTTACGGT	GGATCGGACC	ACCACGCCGC
	TGGCAGACCG	ACGAGCTCCC	CTCAATGCCA	CCTAGCCTGG	TGGTGCGGCG
6951				GCGGTCGGAG	
	CGCTCGGGTT	TCAGGTCTAC	AGGCGCGCGC	CGCCAGCCTC	GAACTACTGT
7001	ACATCGCGCA	GATGGGAGCT	GTCCATGGTC	TGGAGCTCCC	GCGGCGTCAG
	TGTAGCGCGT	CTACCCTCGA	CAGGTACCAG	ACCTCGAGGG	CGCCGCAGTC
7051	GTCAGGCGGG	AGCTCCTGCA	GGTTTACCTC	GCATAGACGG	GTCAGGGCGC
	CAGTCCGCCC	TCGAGGACGT	CCAAATGGAG	CGTATCTGCC	CAGTCCCGCG
7101	GGGCTAGATC	CAGGTGATAC	CTAATTTCCA	GGGGCTGGTT	GGTGGCGGCG
	CCCGATCTAG	GTCCACTATG	GATTAAAGGT	CCCCGACCAA	CCACCGCCGC
7151	TCGATGGCTT	GCAAGAGGCC	GCATCCCCGC	GGCGCGACTA	CGGTACCGCG
	AGCTACCGAA	CGTTCTCCGG	CGTAGGGGCG	CCGCGCTGAT	GCCATGGCGC
7201	CGGCGGGCGG	TEGECCECEG	GGGTGTCCTT	GGATGATGCA	TCTAAAAGCG
	GCCGCCCGCC	ACCCGGCGCC	CCCACAGGAA	CCTACTACGT	AGATTTTCGC
7251	GTGACGCGGG	CGAGCCCCCG	GAGGTAGGGG	GGGCTCCGGA	CCCGCCGGGA
					GGGCGGCCCT
7301	GAGGGGGCAG	GGGCACGTCG	GCGCCGCGCG	CGGGCAGGAG	CTGGTGCTGC
	CTCCCCCGTC	CCCGTGCAGC	CGCGGCGCGC	GCCCGTCCTC	GACCACGACG
7351	GCGCGTAGGT	TGCTGGCGAA	CGCGACGACG	CGGCGGTTGA	TCTCCTGAAT
	CGCGCATCCA	ACGACCGCTT	GCGCTGCTGC	GCCGCCAACT	AGAGGACTTA
7401	CTGGCGCCTC	TGCGTGAAGA	CGACGGGCCC	GGTGAGCTTG	AACCTGAAAG
	GACCGCGGAG	ACGCACTTCT	GCTGCCCGGG	CCACTCGAAC	TTGGACTTTC
7451	AGAGTTCGAC	AGAATCAATT	TCGGTGTCGT	TGACGGCGGC	CTGGCGCAAA
	TCTCAAGCTG	TCTTAGTTAA	AGCCACAGCA	ACTGCCGCCG	GACCGCGTTT

Figure 27H

7501	ATCTCCTGCA TAGAGGACGT	CTCTCTGA GAGGACT	GTTGTCTTGA CAACAGAACT	TAGGCGATON ATCCGCTAGA	GCCGGT. TT
7551			GGAGATCTCC CCTCTAGAGG		
7601	TGGCGGCGAG	GTCGTTGGAA	ATGCGGGCCA	TGAGCTGCGA	GAAGGCGTTG
	ACCGCCGCTC	CAGCAACCTT	TACGCCCGGT	ACTCGACGCT	CTTCCGCAAC
7651			GCGGCTGTAG CGCCGACATC		
7701	CGCCCGCGC	ATGACCACCT TACTGGTGGA	GCGCGAGATT CGCGCTCTAA	GAGCTCCACG CTCGAGGTGC	TGCCGGGCGA ACGGCCCGCT
7751	AGACGGCGTA TCTGCCGCAT	GTTTCGCAGG CAAAGCGTCC	CGCTGAAAGA GCGACTTTCT	GGTAGTTGAG CCATCAACTC	GGTGGTGGCG CCACCACCGC
7801			GTACATAACC		•
7001	CACACAAGAC	GGTGCTTCTT	CATGTATTGG	GTCGCAGCGT	TGCACCTAAG
7851	GTTGATATCC CAACTATAGG	CCCAAGGCCT GGGTTCCGGA	CAAGGCGCTC GTTCCGCGAG	CATGGCCTCG GTACCGGAGC	TAGAAGTCCA ATCTTCAGGT
7901			GAGTTGCGCG		
	GCCGCTTCAA	CTTTTTGACC	CTCAACGCGC	GGCTGTGCCA	ATTGAGGAGG
7951			GGCGACAGTG CCGCTGTCAC		
8001	GGCTACAGGG	GCCTCTTCTT	CTTCTTCAAT	CTCCTCTTCC	ATAAGGGCCT
			GAAGAAGTTA		•
8051	CCCCTTCTTC GGGGAAGAAG	TTCTTCTGGC AAGAAGACCG	GCCGCTGGGG	GAGGGGGGAC CTCCCCCTG	ACGGCGGCGA TGCCGCCGCT
8101	CGACGGCGCA	CCGGGAGGCG	GTCGACAAAG	CGCTCGATCA	TCTCCCCGCG
			CAGCTGTTTC		
8151			TGACGGCGCG ACTGCCGCGC		
8201			ATGTCCCGGT TACAGGGCCA		
8251	CCATGCGGCA GGTACGCCGT	GGGATACGGC CCCTATGCCG	GCTAACGATG CGATTGCTAC	CATCTCAACA GTAGAGTTGT	ATIGTTGTGT TAACAACACA
8301	AGGTACTCCG TCCATGAGGC	CCGCCGAGGG GGCGGCTCCC	ACCTGAGCGA TGGACTCGCT	GTCCGCATCG CAGGCGTAGC	ACCGGATCGG TGGCCTAGCC
8351	AAAACCTCTC TTTTGGAGAG	GAGAAAGGCG CTCTTTCCGC	TCTAACCAGT AGATTGGTCA	CACAGTCGCA GTGTCAGCGT	AGGTAGGCTG TCCATCCGAC
8401	AGCACCGTGG TCGTGGCACC	CGGGCGGCAG GCCCGCCGTC	GCCGCCGCC	TCGGGGTTGT AGCCCCAACA	TTCTGGCGGA AAGACCGCCT

Figure 27I

8451		TACTACATTA			
8501		CACCATGTCC GTGGTACAGG			
8551		CCCAGGCTTC			
8601		AGCCTTTCTA TCGGAAAGAT			
8651		TGCATCTATC ACGTAGATAG			
8701		TTCCTCCCAT AAGGAGGGTA			
8751		AGGTCGCCGA TCCAGCCGCT			
8801					GCGGTGGTAT. CGCCACCATA
8851		TGATGGTGTA ACTACCACAT			
8901		CCCGGCTGCG			
8951		AAATACGTAG TTTATGCATC	-		
9001		AGTGCGGCGG TCACGCCGCC			
9051		CCGGGGGGGA GGCCCCCGCT			TGATATCCGT ACTATAGGCA
9101		GGACATCCAG CCTGTAGGTC			
9151					AAAAGTGCTC TTTTCACGAG
9201					TTGACGCTCT AACTGCGAGA
9251	AGACCGTGCA TCTGGCACGT	AAAGGAGAGC TTTCCTCTCG	CTGTAAGCGG GACATTCGCC	GCACTCTTCC CGTGAGAAGG	CTGGTCTGGT CACCAGACCA
9301	GGATAAATTC CCTATTTAAG	GCAAGGGTAT CGTTCCCATA	CATGGCGGAC GTACCGCCTG	GACCGGGGTT CTGGCCCCAA	CGAGCCCCGT GCTCGGGGCA
9351	ATCCGGCCGT TAGGCCGGCA	CCGCCGTGAT GGCGGCACTA	CCATGCGGTT GGTACGCCAA	ACCGCCCGCG TGGCGGGGCGC	TGTCGAACCC ACAGCTTGGG

Figure 27J

9401	AGGTGTGCGA TCCACACGCT	GEAGTCTGTT	CGGGGGACTG GCCCCCTCAC	CTCCTTTTGG GAGGAAAACC	CTTCCT A GAAGGAAGGT
9451	CCCCCCCCCC	CTGCTGCGCT GACGACGCGA			
9501	TAAGCGGTTA ATTCGCCAAT	GGCTGGAAAG CCGACCTTTC			
9551		ATTTTCCAAG TAAAAGGTTC			
9601	TCGGACCGGC AGCCTGGCCG	CGGACTGCGG GCCTGACGCC			
9651		GCAAATTCCT CGTTTAAGGA			
9701	TTTCCCAGAT AAAGGGTCTA	GCATCCGGTG CGTAGGCCAC			
9751		AAGAGCAGCG TTCTCGTCGC			
9801	TACCGCGTCA ATGGCGCAGT	GGAGGGGCGA CCTCCCGGCT			
9851		CCCCCCCCCC			
9901		TGGCGCGGCT ACCGCGCCGA			
9951		AAGCGTGATA TICGCACTAT			
10001		CCGCGAGGGA GGCGCTCCCT			
10051		GGCGCGAGCT CCGCGCTCGA			
10101		GACTTTGAGC CIGAAACTCG	•		
10151	GCGCACACGT CGCGTGTGCA	CCGCCGGCGG	GACCTGGTAA CTGGACCATT	CCGCATACGA GGCGTATGCT	GCAGACGGTG CGTCTGCCAC
10201	AACCAGGAGA TTGGTCCTCT	TTAACTTTCA AATTGAAAGT			
	TGTGGCGCGC ACACCGCGCG				
10301	TAAGCGCGCT ATTCGCGCGA	GGAGCAAAAC CCTCGTTTTG	CCAAATAGCA GGTTTATCGT	AGCCGCTCAT TCGGCGAGTA	GGCGCAGCTG CCGCGTCGAC

Figure 27K

10351	TTCCTTATAG AAGGAATATC	T GCACAG ACTICCTCTC	CAGGGACAAC GTCCCTGTTG	GAGGCATTCA CTCCGTAAGT	GGGATG T CCCTACGEGA
10401	GCTAAACATA CGATTTGTAT	GTAGAGCCCG CATCTCGGGC	AGGGCCGCTG TCCCGGCGAC	GCTGCTCGAT CGACGAGCTA	TTGATAAACA AACTATTTGT
10451	TCCTGCAGAG AGGACGTCTC	CATAGTGGTG GTATCACCAC	CAGGAGCGCA GTCCTCGCGT	GCTTGAGCCT CGAACTCGGA	GGCTGACAAG CCGACTGTTC
10501	GTGGCCGCCA CACCGGCGGT	TCAACTATTC AGTTGATAAG	CATGCTTAGC GTACGAATCG	CTGGGCAAGT GACCCGTTCA	TTTACGCCCG AAATGCGGGC
10551	CAAGATATAC GTTCTATATG	CATACCCCTT GTATGGGGAA	ACGTTCCCAT TGCAAGGGTA	AGACAAGGAG TCTGTTCCTC	GTAAAGATCG CATTTCTAGC
10601	AGGGGTTCTA TCCCCAAGAT	CATGCGCATG GTACGCGTAC	GCGCTGAAGG CGCGACTTCC	TGCTTACCTT ACGAATGGAA	GAGCGACGAC CTCGCTGCTG
10651	GACCCGCAAA	TAGCGTTGCT	GCGCATCCAC CGCGTAGGTG	TTCCGGCACT	CGCACTCGGC
10701	CGCCGCGCTC	GAGTCGCTGG		CGTGTCGGAC	STTTCCCGGG
10751		CCCGTCGCCG	CTATCTCTCC	GGCTCAGGAT	GAAACTGCGC
10801	CCGCGACTGG	ACGCGACCCG	GGGTTCGGCT	GCGCGGGACC	AGGCAGCTGG TCCGTCGACC
10851	CCGGCCTGGA	CCCGACCGCC	ACCGTGGGCG	CGCGCGACCG	AACGTCGGCG TTGCAGCCGC
10901	CGCACCTCCT	TATACTGCTC	CTGCTACTCA	TGCTCGGTCT	GGACGCCGAG CCTGCCGCTC
10951	ATGATTCGCC	ACTACAAAGA	CTAGTCTACT	ACGTTCTGCG	AACGGACCCG TTGCCTGGGC
11001	CGCCACGCCC	GCCGCGACGT	CTCGGTCGGC	AGGCCGGAAT	ACTCCÄCGGA TGAGGTGCCT
	GCTGACCGCG	GTCCAGTACC	TGGCGTAGTA	CAGCGACTGA	GCGCGCAATC CGCGCGTTAG
11101	GACTGCGCAA	GGCCGTCGTC	GGCGTCCGGT	TGGCCGAGAG	CGCAATTCTG
11151	CTTCGCCACC	AGGGCCGCGC	GCGTTTGGGG	TGCGTGCTCT	AGGTGCTGGC TCCACGACCG
	CTAGCATTTG	CGCGACCGGC	TTTTGTCCCG	GTAGGCCGGG	GACGAGGCCG CTGCTCCGGC
11251	GCCTGGTCTA CGGACCAGAT	CGACGCGCTG	CTTCAGCGCG GAAGTCGCGC	TGGCTCGTTA ACCGAGCAAT	CAACAGEGGE GTTGTEGEEG

Figure 27L

11301	AACGTGCAGA TTGCACGTCT	CCTGGA	CCGGĆTGGTG GGCCGACCAC	CCCCTACACG	GCGAGG T
11351	GGCGCAGCGT CCGCGTCGCA	GAGCGCGCGC CTCGCGCGCG	AGCAGCAGGG TCGTCGTCCC	CAACCTGGGC GTTGGACCCG	TCCATGGTTG AGGTACCAAC
11401	CACTAAACGC GTGATTTGCG	CTTCCTGAGT GAAGGACTCA	ACACAGCCCG TGTGTCGGGC	CCAACGTGCC GGTTGCACGG	CCCCCTGTC
11451	CTCCTGATGT	CCAACTTTGT GGTTGAAACA	CTCGCGTGAC	GCCGATTACC	ACTGACTCTG
11501	TGGCGTTTCA	GAGGTGTACC CTCCACATGG	TCAGACCCGG	TCTGATAAAA	AAGGTCTGGT
11551	CATCTGTTCC	CCTGCAGACC GGACGTCTGG	CATTTGGACT	CCGTCCGAAA	GTTTTTGAAC
11601	GTCCCCGACA	CCCCCCACGC	CCGAGGGTGT	CCGCTGGCGC	GCTGGCACAG
11651	ATCGAACGAC	ACGCCCAACT TGCGGGTTGA	GCGCGGACAA	CGACGACGAT	TATCGCGGGA
11701	TCACGGACAG AGTGCCTGTC	TGGCAGCGTG ACCGTCGCAC	TCCCGGGACA AGGGCCCTGT	CATACCTAGG GTATGGATCC	TCACTTGCTG AGTGAACGAC
11751	ACACTGTACC TGTGACATGG	GCGAGGCCAT CGCTCCGGTA	AGGTCAGGCG TCCAGTCCGC	CATGTGGACG GTACACCTGC	AGCATACTTT TCGTATGAAA
11801	CCAGGAGATT GGTCCTCTAA	ACAAGTGTCA TGTTCACAGT	GCCGCGCGCT CGGCGCGCGA	GGGGCAGGAG CCCCGTCCTC	GACACGGGCA CTGTGCCCGT
11851	GCCTGGAGGC CGGACCTCCG	AACCCTAAAC TTGGGATTTG	TACCTGCTGA ATGGACGACT	CCAACCGGCG GGTTGGCCGC	GCAGAAGATC CGTCTTCTAG
11901	CCCTCGTTGC GGGAGCAACG	ACAGTTTAAA TGTCAAATTT	CAGCGAGGAG	GAGCGCATTT CTCGCGTAAA	TGCGCTACGT ACGCGATGCA
11951	GCAGCAGAGC CGTCGTCTCG	GTGAGCCTTA CACTCGGAAT	ACCTGATGCG TGGACTACGC	CGACGGGGTA GCTGCCCCAT	ACGCCCAGCG TGCGGGTCGC
12001	TGGCGCTGGA ACCGCGACCT	CATGACCGCG GTACTGGCGC	CGCAACATGG GCGTTGTACC	AACCGGGCAT TTGGCCCGTA	GTATGCCTCA CATACGGAGT
12051	AACCGGCCGT TTGGCCGGCA	TTATCAACCG AATAGTTGGC	CCTAATGGAC GGATTACCTG	TACTTGCATC ATGAACGTAG	CGCGCCGCC
12101	CGTGAACCCC GCACTTGGGG	GAGTATTTCA CTCATAAAGT	CCAATGCCAT CCTTACGGTA	CTTGAACCCG GAACTTGGGC	CACTGGCTAC GTGACCGATG
12151	CGCCCCTGG	TTTCTACACC AAAGATGTGG	GGGGGATTCG CCCCTAAGC	AGGTGCCCGA TCCACGGGCT	GGGTAACGAT CCCATTGCTA
12201	GGATTCCTCT CCTAAGGAGA	GGGACGACAT CCCTGCTGTA	AGACGACAGC TCTGCTGTCG	GTGTTTTCCC CACAAAAGGG	CGCAACCGCA GCGTTGGCGT

Figure 27 M

12251	GACCCTGCTA CTGGGACGAT	C. AACGTTG	AGCGCGAGCA TCGCGCTCGT	GGCAGAGGCG CCGTCTCCGC	CCCCTC AA
12301			AGCAGCTTGT TCGTCGAACA		
12351			CCCATTTCCA GGGTAAAGGT		
12401			CGCGCCTGCT GCGCGGACGA		
12451			CAGCGCGAAA GTCGCGCTTT		
12501			CCTAGTGGAC GGATCACCTG		
12551	-		ACGTGCCAGG TGCACGGTCC		
12601			CGGGGTCTGG GCCCCAGACC		
12651			GGATTTGGGA CCTAAACCCT		
12701			GGAGAATGTT CCTCTTACAA		
12751			CAAGGCCATG GTTCCGGTAC		
12801			CCCCCCCCC		
12851			TGAGCGCGGC ACTCGCGCCG		
12901			CTGGACCCGC GACCTGGGCG		
12951			AAACAGCATC TTTGTCGTAG		
13001	CCTATTCGAC GGATAAGCTG		TGTACCTGGT ACATGGACCA		
13051	TGGCATCCCT ACCGTAGGGA				
13101					AGACCATCAA TCTGGTAGTT
13151					ATCCTGCATA TAGGACGTAT

Figure 27N

13201	CCAACATGCC GGTTGTACGG	AZTGTGAAC TTCACTTG	GAGTTCATGT CTCAAGTACA	TTACCAATAA AATGGTTATT	CAAATT
12251	OCCOMO NICO	のこのととととという。	CCCTACTAAC	GACAATCAGG	TGGAGCTGAA
13251	GCCCACTACC	ACAGCGCGAA	CGGATGATTC	CTGTTAGTCC	ACCTCGACTT
13301	ATACGAGTGG	GTGGAGTTCA	CGCTGCCCGA	GGGCAACTAC	TCCGAGACCA
	TATGCTCACC	CACCTCAAGT	GCGACGGGCT	CCCGTTGATG	AGGCTCTGGT
13351	TGACCATAGA	CCTTATGAAC	AACGCGATCG	TEGAGCACTA	CTTGAAAGTG
	ACTGGTATCT	GGAATACTTG	TTGCGCTAGC	ACCTCGTGAT	GAACTTTCAC
				ATCGGGGTAA	አርማምምርአርአር
13401	CCCTCTCTCT	TGCCCCAAGA	CCTTTCGCTG	TAGCCCCATT	TCAAACTGTG
	•				
13451	CCGCAACTTC	AGACTGGGGT	TTGACCCCGT	CACTGGTCTT	GTCATGCCTG
•	GCCGTTGAAG	TCTGACCCCA	AACTGGGGCA	GTGACCAGAA	CAGTACGGAC
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
13301	CCCATATATG	TTTGCTTCGG	AAGGTAGGTC	TGTAGTAAAA	CGACGGTCCT
13551	TGCGGGGTGG	ACTTCACCCA	CAGCCGCCTG	AGCAACTTGT TCGTTGAACA	ACCCCTAGGC
	ACGCCCCACC	TGAAGTGGGT	GTEGGEGAE	ICGIIGAACA	ACCCGIAGGC
13601	CAAGCGGCAA	CCCTTCCAGG	AGGGCTTTAG	GATCACCTAC	GATGATCTGG
	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	CTAGTGGATG	CTACTAGACC
12651	አርርርመርርመል አ	CATTCCCCCA	СФСФФССАТС	TGGACGCCTA	CCAGGCGAGC
13651	TCCCACCATT	GTAAGGGCGT	GAÇAACCTAC	ACCTGCGGAT	GGTCCGCTCG
					•
13701	TTGAAAGATG	ACACCGAACA	GGGCGGGGGT	GGCGCAGGCG	GCAGCAACAG
	AACTTTCTAC	TGTGGCTTGT	CCCGCCCCCA	CCGCGTCCGC	CG1CG11G1C
13751	CAGTGGCAGC	GGCGCGGAAG	AGAACTCCAA	CGCGGCAGCC	GCGGCAATGC
	GTCACCGTCG	CCGCGCCTTC	TCTTGAGGTT	GCGCCGTCGG	CGCCGTTACG
		0010100110	CAMCAMCCCA	TTCGCGGCGA	רארכזידיכרנ
13801				AAGCGCCGCT	
13851	ACACGGGCTG	AGGAGAAGCG	CGCTGAGGCC	GAAGCAGCGG	CCGAAGCTGC
	TGTGCCCGAC	TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	GGCTTCGACG
13901	CCCCCCCT	GCGCAACCCG	AGGTCGAGAA	GCCTCAGAAG	AAACCGGTGA
13301	GCGGGGGCGA	CGCGTTGGGC	TCCAGCTCTT	CGGAGTCTTC	TTTGGCCACT
13951	TCAAACCCCT	GACAGAGGAC	AGCAAGAAAC	GCAGTTACAA	GGATTATTCG
	AGTITGGGGA	CIGICICCIG	1091101119	CGICAMIGII	Gallini
14001	AATGACAGCA	CCTTCACCCA	GTACCGCAGC	TGGTACCTTG	CATACAACTA
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	GTATGTTGAT
14051	CGGCGACCCT	CNGNCCGGNN	かっしていること	GACCCACCCAN	TGCACTCCTC
14021	CCCCCACCCI.	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	ACGTGAGGAC
14101	ACGTAACCTG	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC	AGACATGATG
	TGCATTGGAC	GCCGAGCCTC	GTCCAGATGA	CCAGCAACGG	TCTGTACTAC

Tigure 270

14151	CAAGACCCCG	TOTTCCG	CTCCACGCGC	CAGATCAGCA	ACTTTC T
	GTTCTGGGGC	ACTGGAAGGC	GAGGTGCGCG	GTCTAGTCGT	TGAAAGGCCA
14201		CAGCTGTTGC CTCGACAACG			
14251		CTCCCAACTC CAGGCTTGAG			
14301		TTCCCGAGAA AAGGGCTCTT			
14351	CATCACCACC	GTCAGTGAAA	ACGTTCCTGC	TCTCACAGAT	CACGGGACGC
	GTAGTGGTGG	CAGTCACTTT	TGCAAGGACG	AGAGTGTCTA	GTGCCCTGCG
14401	TACCECTECE	CAACAGCATC	CCACCACAGO	ACCGACTGAC	CATTACTGAC
	ATGGCGACGC	GTTGTCGTAG	CCTCCTCAGO	TCGCTCACTG	GTAATGACTG
14451	GCCAGACGCC	GCACCTGCCC	CTACGTTTAC	AAGGCCCTGG	GCATAGTCTC
	CGGTCTGCGG	CGTGGACGGG	GATGCAAATG	TTCCGGGACC	CGTATCAGAG
14501	GCCGCGCGTC	CTATCGAGCC	GCACTTTTTG	AGCAAGCATG	TCCATCCTTA
	CGGCGCGCAG	GATAGCTCGG	CGTGAAAAAC	TCGTTCGTAC	AGGTAGGAAT
14551	TATCGCCCAG	CAATAACACA	GGCTGGGGCC	TGCGCTTCCC	AAGCAAGATG
	ATAGCGGGTC	GTTATTGTGT	CCGACCCGG	ACGCGAAGGG	TTCGTTCTAC
14601	TTTGGCGGGG AAACCGCCCC	CCAAGAAGCG GGTTCTTCGC	CTCCGACCAA GAGGCTGGTT	CACCCAGTGC GTGGGTCACG	CGCACGCGCC
14651	GCACTACCGC	GCGCCCTGGG	GCGCGCACAA	ACGCGGCCGC	ACTGGGCGCA
	CGTGATGGCG	CGCGGGACCC	CGCGCGTGTT	TGCGCCGGCC	TGACCCGCGT
14701		TGACGCCATC ACTGCGGTAG			
14751	ACGCCCACGC	CGCCACCAGT	GTCCACAGTG	GACGCGGCCA	TTCAGACCGT
	TGCGGGTGCG	GCGGTGGTCA	CAGGTGTCAC	CTGCGCCGGT	AAGTCTGGCA
14801	GGTGCGCGGA CCACGCGCCT	GCCCGGCGCT CGGGCCGCGA	ATGCTAAAAT TACGATTITA	GAAGAGACGG CTTCTCTGCC	CGGAGGCGCG
14851	TAGCACGTCG	CCACCGCCGC	CGACCCGGCA	CTGCCGCCCA	ACGCGCGCGC
	ATCGTGCAGC	GCTGGCGCGCG	GCTGGGCCGT	GACGGCGGGT	TGCGCGCCGC
14901	GCGGCCCTGC CGCCGGGACG	TTAACCGCGC AATTGGCGCG	ACGTCGCACC TGCAGCGTGG	GGCCGACGGG	CGGCCATGCG
14951	GCCCCTCGA	AGGCTGGCCG	CGGGTATTGT	CACTGTGCCC	CCCAGGTCCA
	CCGGCGAGCT	TCGGACCGGC	GCCCATAACA	GTGACACGGG	GGGTCCAGGT
15001	GGCGACGAGC CCGCTGCTCG	GGCCGCCGCA	GCAGCGGGG CCTCGGGGCCC	CCATTAGTGC GGTAATCACG	TATGACTCAG ATACTGAGTC
15051	GGTCGCAGGG	GCAACGTGTA	TTGGGTGCGC	GACTCGGTTA	GCGGCCTGCG
	CCAGCGTCCC	CGTTGCACAT	AACCCACGCG	CTGAGCCAAT	CGCGGACGC

Figure 27P

15101	CGTGCCCGTG GCACGGGCAC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCCCGCGCAA GGGGCGCGTT	CTAGATTGCA GATCTAACGT	AGAAAAA TCTTTTI A
15151			ATGTATCCAG TACATAGGTC		
15201			CAAAGAAGAG GTTTCTTCTC		
15251			AGAAGGAAGA TCTTCCTTCT		
15301			AAAAAGAAAG TTTTTCTCTC		
15351			CGCTACCGCG GCGATGGCGC		
15401	GAAAGGTCGA CTTTCCAGCT	CGCGTAAAAC GCGCATTTTG	GTGTTTTGCG CACAAAACGC	ACCCGGCACC TGGGCCGTGG	ACCGTAGTCT TEGCATCAGA
15451	TTACGCCCGG AATGCGGGCC	TGAGCGCTCC ACTCGCGAGG	ACCCGCACCT TGGGCGTGGA	ACAAGCGCGT TGTTCGCGCA	GTATGATGAG CATACTACTC
15501	CACATGCCGC	TGCTCCTGGA	GCTTGAGCAG CGAACTCGTC	CGGTTGCTCG	CGGAGCCCCT
15551	GTTTGCCTAC CAAACGGATG	GGAAAGCGGC CCTTTCGCCG	ATAAGGACAT TATTCCTGTA	GCTGGCGTTG CGACCGCAAC	CCGCTGGACG GGCGACCTGC
15601	TCCCGTTGGG	TTGTGGATCG	CTAAAGCCCG GATTTCGGGC	ATTGTGACGT	CGTCCACGAC
15651	GGGCGCGAAC	GTGGCAGGCT	AGAAAAGCGC TCTTTTCGCG	CCGGATTTCG	CGCTCAGACC
15701	ACTGAACCGT	GGGTGGCACG	AGCTGATGGT TCGACTACCA	TGGGTTCGCG	GICGCTGACC
15751	TTCTACAGAA	CCTTTTTTAC	ACCGTGGAAC TCGCACCTTG	GACCCGACCT	CGGGCTCCAG
15801	GCGCACGCCG	GTTAGTTCGT	GGTGGCGCCG CCACCGCGGC	CCTGACCCGC	ACGTCTGGCA
15851	CCTGCAAGTC	TATGGGTGAT	CCAGTAGCAC GGTCATCGTG	GTCATAACGG	TGGCGGTGTC
		CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	CCGCCTACGG
		GCCAGCGACG	CCGGCGCAGG	TTCTGGAGAT	GCCTCCACGT
16001	AACGGACCCG TTGCCTGGGC		GCGTTTCAGC CGCAAAGTCG		

Figure 270

16051					TGCCCTAT ACGGGATGTA
16101					ACCGCCCCAG TGGCGGGGTC
16151					66666666666666666666666666666666666666
16201					CAGGGTGGCT GTCCCACCGA
16251		GCAGGACCCT CGTCCTGGGA			ACCACCCCAG TGGTGGGGTC
16301					GCCCTCACCT CGGGAGTGGA
16351	GCCGCCTCCG	TTTCCCGGTG AAAGGGCCAC	CCGGGATTCC GGCCCTAAGG	GAGGAAGAAT CTCCTTCTTA	GCACCGTAGG CGTGGCATCC
16401		CCGGCCACGG GGCCGGTGCC			GTGCGCACCA CACGCGTGGT
16451	CCGCCGCCCC	CGCGCGTCGC GCGCGCAGCG	ACCGTCGCAT TGGCAGCGTA	GCGCGCCGT CGCGCCGCCA	ATCCTGCCCC TAGGACGGGG
16501	TCCTTATTCC AGGAATAAGG	ACTGATCGCC TGACTAGCGG	GCGGCGATTG CGCCGCTAAC	GCGCCGTGCC CGCGGCACGG	CGGAATTGCA GCCTTAACGT
16551		TGCAGGCGCA ACGTCCGCGT			
16601		AATAAAAAGT TTATTTTCA			GGTCCTGTAA CCAGGACATT
16651		GAATGGAAGA CTTACCTTCT			CCCCGCGACA GGGGCGCTGT
16701		CCGTTCATGG GGCAAGTACC			ACCAGCAATA TGGTCGTTAT
16751					CATTAAAAAT GTAATTTTTA
16801					ACAGCAGCAC TGTCGTCGTG
16851	AGGCCAGATG TCCGGTCTAC	CTGAGGGATA GACTCCCTAT			
16901	TGGTAGATGG ACCATCTACC				
16951	CAGGCAGTGC GTCCGTCACG				GCCCTCCCGT CGGGAGGGCA

Figure 27R

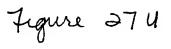
17001					CCCCCACCCC
17051					GCAAATAGAC CGTTTATCTG
17101				CAAGGCCTGC GTTCCGGACG	CCACCACCCG GGTGGTGGGC
17151	TCCCATCGCG AGGGTAGCGC			GGGCCAGCAC CCCGGTCGTG	
17201				AGCAGAAACC TCGTCTTTGG	TGTGCTGCCA ACACGACGGT
17251				AGCCGCGCGT TCGGCGCGCA	
17301				CGTAGCCAGT GCATCGGTCA	
17351				GGGTGCAATC CCCACGTTAG	
17401				ATGTGTGTCA TACACACAGT	
17451				eccececece ccecececcc	
17501				TCTTACATGC AGAATGTACG	
17551				GCTGGTGCAG CGACCACGTC	
17601				AGTTTAGAAA TCAAATCTTT	
17651				TCCCAGCGTT AGGGTCGCAA	
17701	GTTCATCCCT CAAGTAGGGA			GTACTCGTAC CATGAGCATG	
17751	TCACCCTAGC AGTGGGATCG	TGTGGGTGAT ACACCCACTA	AACCGTGTGC TTGGCACACG	TGGACATGGC ACCTGTACCG	TTCCACGTAC AAGGTGCATG
17801	TTTGACATCC AAACTGTAGG				
17851	TGGCACTGCC ACCGTGACGG				
17901	AATGGGATGA TTACCCTACT	AGCTGCTACT TCGACGATGA	GCTCTTGAAA CGAGAACTTT	TAAACCTAGA ATTTGGATCT	AGAAGAGGAC TCTTCTCCTG

Figure 275

17951		A CGAAGT TTCTGCTTCA		
18001		CAGGCGCCTT GTCCGCGGAA		
18051		TGTCGAAGGT ACAGCTTCCA	 	
18101		CTCAAATAGG GAGTTTATCC		
18151		GGGAGAGTCC CCCTCTCAGG	 	
18201		TGCAAAACCC ACGTTTTGGG		
18251		AAAATGGAAA TTTTACCTTT	 	
18301		GAGGCAGCCG CTCCGTCGGC	<del>-</del>	
18351		CAGTGAAGAT GTCACTTCTA		
18401		CCACTATTAA GGTGATAATT	 	
18451		CCCAACAGGC GGGTTGTCCG		
18501		GTATTACAAC CATAATGTTG		
18551		AGTIGAATGC TCAACTTACG	 	
18601		CAGCTTTTGC GTCGAAAACG	 	
18651		GAATCAGGCT CTTAGTCCGA		
18701	ATTGAAAATC TAACTTTTAG	ATGGAACTGA TACCTTGACT	 	
18751	GCGAGGTGTG CCCTCCACAC	ATTAATACAG TAATTATGTC		
18801	GTCAGGAAAA CAGTCCTTTT	TGGATGGGAA ACCTACCCTT		
18851	GAAATAAGAG CTTTATTCTC	TTGGAAATAA AACCTTTATT		

Figure 27T

18901	CCTGTGGAGA GGACACCTCT	A TCCTGT TTAAAGGACA	ACTCCAACAT TGAGGTTGTA	AGCGCTGTAT TCGCGACATA	AACGGGCTGT
18951			AACGTAAAA TTGCATTTTT		
19001	TACGACTACA ATGCTGATGT		AGTGGTGGCT TCACCACCGA		
19051			GGTCCCTTGA CCAGGGAACT		
19101			GCTGGCCTGC CGACCGGACG		
19151			CTTCCACATC GAAGGTGTAG		
19201			TCCTGCCGGG AGGACGGCCC		
19251			ATGGTTCTGC TACCAAGACG		
19301			CATTAAGTTT GTAATTCAAA		
19351			ACAACACCGC TGTTGTGGCG		
19401			CAGTCCTTTA GTCAGGAAAT		
19451			CGCCAACGCT GCGGTTGCGA		
19501			CTTTCCGCGG GAAAGGCGCC		
19551	AGACTAAGGA TCTGATTCCT	AACCCCATCA TTGGGGTAGT	CTGGGCTCGG GACCCGAGCC	GCTACGACCC CGATGCTGGG	TTATTACACC AATAATGTGG
19601			CCTAGATGGA GGATCTACCT		
19651	CTTTAAGAAG GAAATTCTTC	GTGGCCATTA CACCGGTAAT	CCTTTGACTC GGAAACTGAG	TTCTGTCAGC AAGACAGTCG	TGGCCTGGCA ACCGGACCGT
19701	ATGACCGCCT TACTGGCGGA	GCTTACCCCC CGAATGGGGG	AACGAGTTTG TTGCTCAAAC	AAATTAAGCG TTTAATTCGC	CTCAGTTGAC GAGTCAACTG
19751	GGGGAGGGTT CCCCTCCCAA	ACAACGTTGC TGTTGCAACG	CCAGTGTAAC GGTCACATTG	ATGACCAAAG TÄCTGGTTTC	ACTGGTTCCT TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC GATGGTCCCG	TTCTATATCC AAGATATAGG



19851	CAGAGAGCTA GTCTCTCGAT			CTTCCA CCC GAAGGTCGGG
19901			TGATACTAAA ACTATGATTT	 ACCAACAGGT TGGTTGTCCA
19951	GGGCATCCTA CCCGTAGGAT		ACAACTCTGG TGTTGAGACC	 
20001			GCCTACCCTG CGGATGGGAC	CTATCCGCTT GATAGGCGAA
20051	ATAGGCAAGA TATCCGTTCT		CAGCATTACC GTCGTAATGG	
20101			CATTCTCCAG GTAAGAGGTC	 
20151	CACTCACAGA GTGAGTGTCT		AACCTTCTCT TTGGAAGAGA	
20201		-	GGATCCCATG CCTAGGGTAC	CCCTTCTTTA GGGAAGAAAT
20251			ACGTGGTCCG TGCACCAGGC	
20301			CTGCGCACGC GACGCGTGCG	
20351			ACATCAACAA TGTAGTTGTT	
20401			CATTGTCAAA GTAACAGTTT	 GTGGGCCATA CACCCGGTAT
20451			AGCGCTTTCC TCGCGAAAGG	
20501				TGGGGGCGTA ACCCCCGCAT
20551				 GCTACCTCTT CGATGGAGAA
20601	TGAGCCCTTT ACTCGGGAAA			TACCAGTTTG ATGGTCAAAC
20651	AGTACGAGTC TCATGCTCAG			CCCCGACCGC GGGGCTGGCG
20701	TGTATAACGC ACATATTGCG			CCAACTCGGC GGTTGAGCCG
20751	CGCCTGTGGA GCGGACACCT			GCCAACTGGC CGGTTGACCG

Figure 27V

20801	CCCAAACTCC GGGTTTGAGG	C GATCAC GTACCTAGTG	AACCCCACCA TTGGGGTGGT	TGAACCTTAT ACTTGGAATA	TACCGG AT ATGGCCCCAT
20851				CAGCCCACCC GTCGGGTGGG	TGCGTCGCAA ACGCAGCGTT
20901	GGTCCTTGTC	GAGATGTCGA	AGGACCTCGC	CCACTCGCCC GGTGAGCGGG	ATGAAGGCGT
20951	CGGTGTCACG	CGTCTAATCC	TCGCGGTGAA	CTTTTTGTCA GAAAAACAGT	GAACTTTTTG
21001	TACATTTTTA	TTACATGATC	TCTGTGAAAG	AATAAAGGCA TTATTTCCGT	TTACGAAAAT
21051	AAACATGTGA	GAGCCCACTA	ATAAATGGGG	CACCCTTGCC GTGGGAACGG	CAGACGCGGC
21101	AAATTTTTAG	TTTCCCCAAG	ACGGCGCGTA	CGCTATGCGC GCGATACGCG	GTGACCGTCC
21151	CTGTGCAACG	CTATGACCAC	AAATCACGAG	CACTTAAACT GTGAATTTGA	GTCCGTGTTG
21201	GTAGGCGCCG	TCGAGCCACT	TCAAAAGTGA	CCACAGGCTG GGTGTCCGAC	GCGTGGTAGT
21251	GGTTGCGCAA	ATCGTCCAGC	CCGCGGCTAT	TCTTGAAGTC AGAACTTCAG	CGTCAACCCC
21301	GGAGGCGGGA	CGCGCGCGCT	CAACGCTATG	ACAGGGTTGC TGTCCCAACG	TCGTGACCTT
21351	GTGATAGTCG	CGGCCCACCA	CGTGCGACCG	CAGCACGCTC GTCGTGCGAG	AACAGCCTCT
21401	AGTCTAGGCG	CAGGTCCAGG	AGGCGCAACG	TCAGGGCGAA AGTCCCGCTT	GCCTCAGTTG
21451	AAACCATCGA	CGGAAGGGTT	TTTCCCGCGC	TGCCCAGGCT ACGGGTCCGA	AACTCAACGT
21501	GAGCGTGGCA	TCACCGTAGT	TTTCCACTGG	GTGCCCGGTC CACGGGCCAG	ACCCGCAATC
21551		GACGTATTTT	CGGAACTAGA	CGAATTTTCG	GTGGACTCGG
21601	AAACGCGGAA	GTCTCTTCTT	GTACGGCGTT	CTGAACGGCC	AAAACTGATT TTTTGACTAA
	CCGGCCTGTC	CGGCGCAGCA	CETECETCET	GGAACGCAGC	GTGTTGGAGA CACAACCTCT
21701	TCTGCACCAC AGACGTGGTG	ATTTCGGCCC TAAAGCCGGG	CACCGGTTCT GTGGCCAAGA	TCACGATCTT AGTGCTAGAA	GGCCTTGCTA CCGGAACGAT

7, gure 27 W

21751	GACTGCTCCT CTGACGAGGA	T CGCGCG AGTCGCGCGC	CTGCCCGTTT GACGGGCAAA	TCGCTCGTCA AGCGAGCAGT	CATCUATE C GTAGGTAAAG
21801			TCATAATGCT AGTATTACGA		
21851			CGGTGCAGCC GCCACGTCGG		
21901			CTCTGCAAAC GAGACGTTTG		
21951			CAAAGGTCTT GTTTCCAGAA		
22001			TTCAGCCAGG AAGTCGGTCC		
22051			TAGTTTGAAG ATCAAACTTC		
22101	GTGCACCATG	AACAGGTAGT	CGCGCGCGCGC	TCGGAGGTAC	GGGAAGAGGG
22151			CTCAGCGGGT GAGTCGCCCA		
22201			CTCTTCCTCT GAGAAGGAGA		
22251			GCCGCCGCAC CGCCGCGTG		
22301	GTACGAACTA	ATCGTGGCCA	GGGTTGCTGA CCCAACGACT	TTGGGTGGTA	AACATCGCGG
22351			GCTGTCCACG CGACAGGTGC		
22401	CGCGAGCCCG	AACCCTCTTC	GGCGCTTCTT CCGCGAAGAA	AAAGAAGAAC	CCGCGTTACC
22451			GATGGCCGCG CTACCGGCGC		GCGCGCACC
22501	AGCGCGTCTT TCGCGCAGAA				TACGCCGCCT ATGCGGCGGA
22551	CATCCGCTTT GTAGGCGAAA				GGGGACGGGG
	ACGACACGTC TGCTGTGCAG				
22651	TCGGGGGTGG AGCCCCCACC				TTTCCTTCTC AAAGGAAGAG

Figure 27X

22701	CTATACGCAG GATATCCGTC	A AGATCA TTTTTCTAGT	TGGAGTCAGT ACCTCAGTCA	CGAGAAGAAG GCTCTTCTTC	GACAGCO A CTGTCGGATT
22751		TGAGTTCGCC ACTCAAGCGG			
22801		TCCCCGTCGA AGGGGCAGCT			
22851		GACCCAGGTT CTGGGTCCAA			
22901		GGATAAAAAG CCTATTTTTC			
22951		GCCGGGGGA CCGCCCCCT			
23001		CTGTTGAAGC GACAACTTCG			
23051		AGAGCGCAGC TCTCGCGTCG			
23101		AACGCCACCT TTGCGGTGGA			
23151		ACATGCGAGC TGTACGCTCG			
23201		AGAGGTGCTT TCTCCACGAA			
23251		TATCCTGCCG ATAGGACGGC			
23301		CAGGGCGCTG GTCCCGCGAC	-		
23351		CTTTGAGGGT GAAACTCCCA			
23401		AGGAAAACAG TCCTTTTGTC			
23451	GGAACTCGAG CCTTGAGCTC	GGTGACAACG CCACTGTTGC	CGCGCCTAGC GCGCGGATCG	CGTACTAAAA GCATGATTTT	CGCAGCATCG GCGTCGTAGC
23501	AGGTCACCCA TCCAGTGGGT	CTTTGCCTAC GAAACGGATG			
23551	AGCACAGTCA TCGTGTCAGT	TGAGTGAGCT ACTCACTCGA	GATCGTGCGC CTAGCACGCG	CGTGCGCAGC GCACGCGTCG	CCCTGGAGAG GGGACCTCTC
23601	GGATGCAAAT CCTACGTTTA	TTGCAAGAAC AACGTTCTTG			

Figure 27 Y

23651	ACGAGCAGCT TGCTCGTCGA	A CGCTGG TCGCGCGACC		
23701		AACTAAȚGAT TTGATTACTA	 	
23751		CGCTTCTTTG GCCAAGAAAC		
23801		CTACACCTTT GATGTGGAAA		
23851		TGGAGCTCTG ACCTCGAGAC		
23901		CTTGGGCAAA GAACCCGTTT	 	
23951		CTACGTCCGC GATGCAGGCG	 	
24001		CCATGGGCGT GGTACCCGCA		
24051		CAGAAACTGC GTCTTTGACG		
24101		GCGCTCCGTG CGCGAGGCAC	 	
24151		TTAAAACCCT AATTTTGGGA		
24201		CAGAACTTTA GTCTTGAAAT		
24251		CTGCTGTGCA GACGACACGT		
24301		CTCCGCCGCT GAGGCGGCGA		
24351		GCCTACCACT CGGATGGTGA		
24401	GTCTACTGGA CAGATGACCT	CTCTCACTCT CACAGTGACA		
24451	CTGGTTTGCA GACCAAACGT			
	TGAGCTGCAG ACTCGACGTC			
24551	AACTCACTCC TTGAGTGAGG			

Figure 27Z

24601	GAGGACTACC				
	CTCCTGATGG	TGCGGGTGCT	CIMATCCAAG	AIGCTTCTGG	TAGGGCGGG
24651	GCCTAATGCG	GAGCTTACCG	CCTGCGTCAT	TACCCAGGGC	CACATTCTTG
	CGGATTACGC	CTCGAATGGC	GGACGCAGTA	ATGGGTCCCG	GTGTAAGAAC
24701		AGCCATCAAC			
	CGGTTAACGT	TCGGTAGTTG	TTTCGGGCGG	TICTCAAAGA	CGATGCTTTC
24751	GGACGGGGG	TTTACTTGGA	CCCCCAGTCC	GGCGAGGAGC	TCAACCCAAT
	CCTGCCCCCC	AAATGAACCT	GGGGGTCAGG	CCGCTCCTCG	AGTTGGGTTA
24801		CCGCAGCCCT			
	GGGGGGGGG	GGCGTCGGGA	TAGTCGTCGT	CGGCGCCCGG	GAACGAAGGG
24851	AGGATGGCAC	CCAAAAAGAA	GCTGCAGCTG	CCGCCGCCAC	CCACGGACGA
	•••	GGTTTTTCTT			
24901		TGGGACAGTC			
	CCTCCTTATG	ACCCTGTCAG	TCCGTCTCCT	CCAAAACCTG	CTCCTCCTCC
24951	AGGACATGAT	GGAAGACTGG	GAGAGCCTAG	ACGAGGAAGC	TTCCGAGGTC
*****		CCTTCTGACC			
25001	0.2.00.00.00	CAGACGAAAC			
	CTTCTCCACA	GTCTGCTTTG	TGGCAGTGGG	AGCCAGCGTA	AGGGGAGCGG
25051	cececcese	AAATCGGCAA	CCGCTTCCAG	CATGGCTACA	ACCTCCGCTC
23031		TTTAGCCGTT			
				V 100	
25101	CTCAGGCGCC	GCCGGCACTG	CCCGTTCGCC	GACCCAACCG	TAGATGGGAC
	GAGTCCGCGG	CGGCCGTGAC	GGGCAAGCGG	CTGGGTTGGC	ATCTACCCTG
05151		CCAGGGCCGG			CCTTA CCCCA
25151	***	GGTCCCGGCC			
	1661640011	36100000		010000000	0
25201	AGAGCAACAA	CAGCGCCAAG	GCTACCGCTC	ATGGCGCGGG	CACAAGAACG
	TCTCGTTGTT	GTCGCGGTTC	CGATGGCGAG	TACCGCGCCC	GTGTTCTTGC
			G1 0000000	001101momo	0mm0000000
25251		TTGCTTGCAA AACGAACGTT			
	GGIMICANCE	AACGAACGII	CIGACACCC	CGIIGIAGAG	GAAGC GGGCG
25301	CGCTTTCTTC	TCTACCATCA	CGGCGTGGCC	TTCCCCCGTA	ACATCCTGCA
	GCGAAAGAAG	AGATGGTAGT	GCCGCACCGG	AAGGGGGCAT	TGTAGGACGT
				*********	
25351	TTACTACCGT	GTAGAGATGT			
	AATGATGGCA	GIAGAGAIGI	CGGGIAIGAC	0100000000	1000031031
25401	ACAGCAGCGG	CCACACAGAA	GCAAAGGCGA	CCGGATAGCA	AGACTCTGAC
		GGTGTGTCTT			
25451	AAAGCCCAAG				
	TTTCGGGTTC	TTTAGGTGTC	GUCGCCGTCG	TEGICETECT	CCTCGCGACG
25501	GTCTGGCGCC	CAACGAACCC	GTATCGACCC	GCGAGCTTAG	AAACAGGATT
		GTTGCTTGGG			

Figure 27. AA

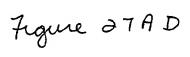
25551	TTTCCCACTC	TO TGCTAT	ATTTCAACAG TAAAGTTGTC	AGCAGGGGCC TCGTCCCCGG	AAGAACATA TTCTTGTTCT
25601					
			GAGACGCTAG		
25651	ATCACAAAAG TAGTGTTTTC		CTTCGGCGCA GAAGCCGCGT		
25701			GCTGACTCTT CGACTGAGAA		
25751			AACTACGTCA TTGATGCAGT		
25801	CGCCAGCACC	ACAAÇAGTCG	GCCATTATGA	GCAAGGAAAT	TCCCACGCCC
	GCGGTCGTGG	TGTTGTCAGC	CGGTAATACT	CGTTCCTTTA	AGGGTGCGGG
25851	TACATGTGGA	GTTACCAGCC	ACAAATGGGA	CTTGCGGCTG	GAGCTGCCCA
	ATGTACACCT	CAATGGTCGG	TGTTTACCCT	GAACGCCGAC	CTCGACGGGT
25901	AGACTACTCA	ACCCGAATAA	ACTACATGAG	CGCGGGACCC	CACATGATAT
	TCTGATGAGT	TGGGCTTATT	TGATGTACTC	GCGCCCTGGG	GTGTACTATA
25951	CCCGGGTCAA	CGGAATACGC	GCCCACCGAA	ACCGAATTCT	CCTGGAACAG
	GGGCCCAGTT	GCCTTATGCG	CGGGTGGCTT	TGGCTTAAGA	GGACCTTGTC
26001	GCGGCTATTA	CCACCACACC	TCGTAATAAC	CTTAATCCCC	GTAGTTGGCC
	CGCCGATAAT	GGTGGTGTGG	AGCATTATTG	GAATTAGGGG	CATCAACCGG
26051	CGCTGCCCTG	GTGTACCAGG	AAAGTCCCGC	TCCCACCACT	GTGGTACTTC
	GCGACGGGAC	CACATGGTCC	TTTCAGGGCG	AGGGTGGTGA	CACCATGAAG
26101	CCAGAGACGC	CCAGGCCGAA	GTTCAGATGA	CTAACTCAGG	GGCGCAGCTT
	GGTCTCTGCG	GGTCCGGCTT	CAAGTCTACT	GATTGAGTCC	CCGCGTCGAA
26151	GCGGGCGGCT	TTCGTCACAG	GGTGCGGTCG	CCCGGGCAGG	GTATAACTCA
	CGCCCGCCGA	AAGCAGTGTC	CCACGCCAGC	GGGCCCGTCC	CATATTGAGT
26201	CCTGACAATC	AGAGGGCGAG	GTATTCAGCT	CAACGACGAG	TCGGTGAGCT
	GGACTGTTAG	TCTCCCGCTC	CATAAGTCGA	GTTGCTGCTC	AGCCACTCGA
26251	CCTCGCTTGG	TCTCCGTCCG	GACGGGACAT	TŢCAGATCGG	600606606
	GGAGCGAACC	AGAGGCAGGC	CTGCCCTGTA	AAGTCTAGCC	6006066666
26301	CGCTCTTCAT	TCACGCCTCG	TCAGGCAATC	CTAACTCTGC	AGACCTCGTC
	CCGAGAAGTA	AGTGCGGAGC	AGTCCGTTAG	GATTGAGACG	TCTGGAGCAG
26351	CTCTGAGCCG	CGCTCTGGAG	GCATTGGAAC	TCTGCAATTT	ATTGAGGAGT
	GAGACTCGGC	GCGAGACCTC	CGTAACCTTG	AGACGTTAAA	TAACTCCTCA
26401	TTGTGCCATC AACACGGTAG	GGTCTACTTT CCAGATGAAA	AACCCCTTCT TTGGGGAAGA	CGGGACCTCC	CGGCCACTAT GCCGGTGATA
26451	CCGGATCAAT GGCCTAGTTA	TTATTCCTAA AATAAGGATT	CTTTGACGCG GAAACTGCGC	GTAAAGGACT CATTTCCTGA	CGGCGGACGG

Figure 27 AB

26501	GATGCTGACT	A TAAGTG TACAATTCAC	CTCTCCGTCT	CGTTGACGCG	GACTTTGTGG
26551	TGGTCCACTG	TCGCCGCCAC	AAGTGCTTTG	CCCGCGACTC	CGGTGAGTTT
	ACCAGGTGAC	AGCGGCGGTG	TTCACGAAAC	GGGCGCTGAG	GCCACTCAAA
26601		AATTGCCCGA TTAACGGGCT			
26651		GCCCAGGGAG CGGGTCCCTC			
26701		CCTGCTAGTT GGACGATCAA			
26751	GTGATTTGCA	ACTGTCCTAA	CCCTGGATTA	CATCAAGATC	TTTGTTGCCA
	CACTAAACGT	TGACAGGATT	GGGACCTAAT	GTAGTTCTAG	AAACAACGGT
26801	TCTCTGTGCT	GAGTATAATA	AATACAGAAA	ATARAATTA	CTGGGGCTCC
	AGAGACACGA	CTCATATTAT	TTATGTCTTT	TATATTTAA	GACCCCGAGG
26851	TATCGCCATC	CTGTAAACGC	CACCGTCTTC	ACCCGCCCAA	GCAAACCAAG
	ATAGCGGTAG	GACATTTGCG	GTGGCAGAAG	TGGGCGGGTT	CGTTTGGTTC
26901	GCGAACCTTA	CCTGGTACTT	TTAACATCTC	TCCCTCTGTG	ATTTACAACA
	CGCTTGGAAT	GGACCATGAA	AATTGTAGAG	AGGGAGACAC	TAAATGTTGT
26951	GTTTCAACCC	AGACGGAGTG	AGTCTACGAG	AGAACCTCTC	CGAGCTCAGC
	CAAAGTTGGG	TCTGCCTCAC	TCAGATGCTC	TCTTGGAGAG	GCTCGAGTCG
27001	TACTCCATCA	GAAAAAACAC	CACCCTCCTT	ACCTGCCGGG	AACGTACGAG
	ATGAGGTAGT	CTTTTTTGTG	GTGGGAGGAA	TGGACGGCCC	TTGCATGCTC
27051	TGCGTCACCG	GCCGCTGCAC	CACACCTACC	GCCTGACCGT	AAACCAGACT
	ACGCAGTGGC	CGGCGACGTG	GTGTGGATGG	CGGACTGGCA	TTTGGTCTGA
27101	TTTTCCGGAC	AGACCTCAAT	AACTCTGTTT	ACCAGAACAG	GAGGTGAGCT
	AAAAGGCCTG	TCTGGAGTTA	TTGAGACAAA	TGGTCTTGTC	CTCCACTCGA
27151	TAGAAAACCC ATCTTTTGGG	TTAGGGTATT AATCCCATAA	AGGCCAAAGG TCCGGTTTCC	CCCACCTACT GCGTCGATGA	CACCCCAAAT
27201	TGAACAATTC	AAGCAACTCT	ACGGGCTATT	CTAATTCAGG	TTTCTCTAGA
	ACTTGTTAAG	TTCGTTGAGA	TGCCCGATAA	GATTAAGTCC	AAAGAGATCT
27251	ATCGGGGTTG	GGGTTATTCT	CTGTCTTGTG	ATTCTCTTTA	TTCTTATACT
	TAGCCCCAAC	CCCAATAAGA	GACAGAACAC	TAAGAGAAAT	AAGAATATGA
27301	AACGCTTCTC	TGCCTAAGGC	TCGCCGCCTG	CTGTGTGCAC	ATTTGCATTT
	TTGCGAAGAG	ACGGATTCCG	AGCGGCGGAC	GACACACGTG	TAAACGTAAA
27351	ATTGTCAGCT	TTTTAAACGC	TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA
	TAACAGTCGA	AAAATTTGCG	ACCCCAGCGG	TGGGTTCTAC	TAATCCATGT
27401	TAATCCTAGG	TTTACTCACC	CTTGCGTCAG	CCCACGGTAC	CACCCAAAAG
	ATTAGGATCC	AAATGAGTGG	GAACGCAGTC	GGGTGCCATG	GTGGGTTTTC

Figure 27AC

27451	GTGGATTTTA	A COCAGO	ርብረታው ያ ያለርነውው	<b>እ</b> ርልሞምርርርልር	СТСААС
21431		TCCTCGGTCG			_
			•		
27501	- · <del>-</del>	ACTOTTATAA			
	ACTCACGTGG	TGAGAATATT	TTACGTGGTG	TCTTGTACTT	TTCGACGAAT
27551	TTCGCCACAA	AAACAAAATT	GGCAAGTATG	CTGTTTATGC	TATTTGGCAG
		TTTGTTTTAA			
27601		CTACAGAGTA			
	GGTCCACTGT	GATGTCTCAT	ATTACAATGT	CAAAAGGICC	CATTTTCAGT
27651	TAAAACTTTT	ATGTATACTT	TTCCATTTTA	TGAAATGTGC	GACATTACCA
	ATTTTGAAAA	TACATATGAA	AAGGTAAAAT	ACTTTACACG	CTGTAATGGT
	·				
27701		CAAACAGTAT			
	ACAIGIACIC	GITIGICAIA	TTCAACACCG	GGGGTGTTTT	AACACACCII
27751		CTTTCTGCTG			
	TTGTGACCGT	GAAAGACGAC	GTGACGATAC	GATTAATGTC	ACGAGCGAAA
					3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
27801		CTACTCTATA GATGAGATAT			
	CCAGACA166	GAIGAGAIAI	AATTIAIGIT	110010100	ICOMMIANC
27851	AGGAAAAGAA	AATGCCTTAA	TTTACTAAGT	TACAAAGCTA	ATGTCACCAC
	TCCTTTTCTT	TTACGGAATT	AAATGATTCA	ATGTTTCGAT	TACAGTGGTG
		ACTCGCTGCT	mac	>mma>>>>0	mm>CC>mm>M
27901		TGAGCGACGA			
	ATTOACGAAA	IGNOCUNCON	ACCITION		
27951		GGATTTAAAC			
	TTAATCTTAT	CCTAAATTTG	GGGGGCCAGT	AAAGGACGAG	TTÄTGGTAAG
28001	CCCMCAACAA	TTGACTCTAT	CTCCCTTTTC	ריזירים ברכבריזי	<b>እ</b> ርგგርርተማርል
28001		AACTGAGATA			
28051		CCTGGATGTC			
	TCAGTCCGAA	GGACCTACAG	TCGTAGACTG	AAACCGGTCG	TGGACAGGGC
28101	೧೯೯ <b>೨ ಪಿಸುಗು</b> ರವರ್ಗ	CCAGTCCAAC	TACAGCGACC	CACCCTAACA	GAGATGACCA
20101		GGTCAGGTTG			
28151					CACAAATACA
	TGTGTTGGTT	GCGCCGGCGG	CGATGGCCTG	AATGTAGATG	GTGTTTATGT
28201	CCCCAAGTTT	CTGCCTTTGT	CAATAACTGG	GATAACTTGG	GCATGTGGTG
					CGTACACCAC
28251					TGGCTCATCT
	CAAGAGGTAT	CGCGAATACA	AACATACGGA	ATAATAATAC	ACCGAGTAGA
283.01	GCTGCCTAAA	GCGCAAACGC	GCCCGACCAC	CCATCTATAG	TCCCATCATT
	CGACGGATTT	CGCGTTTGCG	CGGGCTGGTG	GGTAGATATC	AGGGTAGTAA
28351	GTGCTACACC	CAAACAATGA	TGGAATCCAT	AGATTGGACG	GACTGAAACA
	CACGATGTGG	GTTTGTTACT	ACCTTAGGTA	TCTAACCTGC	CTGACTTTGT



28401	CATGTTCTTT	TTACAG	TATGATTAAA	TGAGACATGÃ	TTCCTC T
	GTACAAGAAA	AGAGAATGTC	ATACTAATTT	ACTCTGTACT	AAGGAGCTCA
28451	TTTTATATTA	CTGACCCTTG	TTGCGCTTTT	TTGTGCGTGC	TCCACATTGG
	AAAATATAAT	GACTGGGAAC	AACGCGAAAA	AACACGCACG	AGGTGTAACC
28501	CTGCGGTTTC	TCACATCGAA	GTAGACTGCA	TTCCAGCCTT	CACAGTCTAT
	GACGCCAAAG	AGTGTAGCTT	CATCTGACGT	AAGGTCGGAA	GTGTCAGATA
28551	TTGCTTTACG	GATTTGTCAC	CCTCACGCTC	ATCTGCAGCC	TCATCACTGT
	AACGAAATGC	CTAAACAGTG	GGAGTGCGAG	TAGACGTCGG	AGTAGTGACA
28601	GGTCATCGCC	TTTATCCAGT	GCATTGACTG	GGTCTGTGTG	CGCTTTGCAT
	CCAGTAGCGG	AAATAGGTCA	CGTAACTGAC	CCAGACACAC	GCGAAACGTA
28651	ATCTCAGACA	CCATCCCCAG	TACAGGGACA	GGACTATAGC	TGAGCTTCTT
	TAGAGTCTGT	GGTAGGGGTC	ATGTCCCTGT	CCTGATATCG	ACTCGAAGAA
28701	AGAATTCTTT	AATTATGAAA	TTTACTGTGA	CTTTTCTGCT	GATTATTTGC
	TCTTAAGAAA	TTAATACTTT	AAATGACACT	GAAAAGACGA	CTAATAAACG
28751	ACCCTATCTG	CGTTTTGTTC	CCCGACCTCC	AAGCCTCAAA	GACATATATC
	TGGGATAGAC	GCAAAACAAG	GCGCTGGAGG	TTCGGAGTTT	CTGTATATAG
28801	ATGCAGATTC	ACTCGTATAT	GGAATATTCC	AAGTTGCTAC	AATGAAAAAA
	TACGTCTAAG	TGAGCATATA	CCTTATAAGG	TTCAACGATG	TTACTTTTTT
28851	GCGATCTTTC	CGAAGCCTGG	TTATATGCAA	TCATCTCTGT	TATGGTGTIC
	CGCTAGAAAG	GCTTCGGACC	AATATACGTT	AGTAGAGACA	ATACCACAAG
28901	TGCAGTACCA	TCTTAGCCCT	AGCTATATAT	CCCTACCTTG	ACATTGGCTG
	ACGTCATGGT	AGAATCGGGA	TCGATATATA	GGGATGGAAC	TGTAACCGAC
28951	GAACGCAATA	GATGCCATGA	ACCACCCAAC	TTTCCCCGCG	CCCGCTATGC
	CTTGCGTTAT	CTACGGTACT	TGGTGGGTTG	AAAGGGGCGC	GGGCGATACG
29001	TTCCACTGCA AAGGTGACGT	ACAAGTTGTT TGTTCAACAA	GCCGGCGGCT	TTGTCCCAGC AACAGGGTCG	CAATCAGCCT GTTAGTCGGA
29051	CGCCCACCTT	CTCCCACCC	CACTGAAATC	AGCTACTTTA	ATCTAACAGG
	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAT	TAGATTGTCC
29101	AGGAGATGAC	TGACACCCTA	GATCTAGAAA	TGGACGGAAT	TATTACAGAG
	TCCTCTACTG	ACTGTGGGAT	CTAGATCTTT	ACCTGCCTTA	ATAATGTCTC
29151	CAGCGCCTGC	TAGRARGACG	CAGGGCAGCG	GCCGAGCAAC	AGCGCATGAA
	GTCGCGGACG	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	TCGCGTACTT
29201	TCAAGAGCTC	CAAGACATGG	TTAACTTGCA	CCAGTGCAAA	AGGGGTATCT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	TCCCCATAGA
29251	TTTGTCTCGT	AAAGCAGGCC	AAAGTCACCT	ACGACAGTAA	TACCACCGGA
	AAACAGAGCA	TTTCGTCCGG	TTTCAGTGGA	TGCTGTCATT	ATGGTGGCCT
29301	CACCGCCTTA	GCTACAAGTT	GCCAACCAAG	CGTCAGAAAT	TGGTGGTCAT
	GTGGCGGAAT	CGATGTTCAA	CGGTTGGTTC	GCAGTCTTTA	ACCACCAGTA

Figure 27 AE

29351	GCTGGGAGAA CCACCCTCTT	A CCATTA TTCGGGTAAT	CCATAACTCA GGTATTGAGT	GCACTCGGTA CGTGAGCCAT	GAAACCTCC CTTTGGCTTC
29401				AGGATCTCTG TCCTAGAGAC	
29451				CCCTTTAACT GGGAAATTGA	
29501				TTAGCAAATT AATCGTTTAA	
29551				CAGCTCTGGT GTCGAGACCA	
29601				AAATGGAATG TTTACCTTAC	
29651				TCATGTTGTT AGTACAACAA	
29701				CCCGTGTATC GGGCACATAG	
29751				TACTCCTCCC ATGAGGAGGG	
29801				TACTCTCTTT ATGAGAGAAA	
29851				GCGCTCAAAA CGCGAGTTTT	
29901	CCTCTCTCTG GGAGAGAGAC	GACGAGGCCG CTGCTCCGGC	GCAACCTTAC CGTTGGAATG	CTCCCAAAAT GAGGGTTTTA	GTAACCACTG CATTGGTGAC
29951	TGAGCCCACC	TCTCAAAAAA	ACCAAGTCAA	ACATAAACCT	GGAAATATCT CCTTTATAGA
30001	GCACCCCTCA	CAGTTACCTC	AGAAGCCCTA	ACTGTGGCTG	CCGCCGCACC GGCGGCGTGG
30051	TCTAATGGTC	GCGGGCAACA	CACTCACCAT	GCAATCACAG	GCCCCGCTAA CGGGGCGATT
30101	CCGTGCACGA	CTCCAAACTT	AGCATTGCCA	CCCAAGGACC	CCTCACAGTG GGAGTGTCAC
30151	TCAGAAGGAA	AGCTAGCCCT	GCAAACATCA	GCCCCCTCA	CCACCACCGA GGTGGTGGCT
30201	TAGCAGTACC	CTTACTATCA	CTGCCTCACC	CCCTCTAACT	ACTGCCACTG TGACGGTGAC
30251	GTAGCTTGGG	CATTGACTTG	AAAGAGCCCA	TTTATACACA	AAATGGAAAA TTTACCTTTT

Figure 27 AF

30301	CTAGGACTAA GATCCTGATT	A CGGGGC	TCCTTTGCAT AGĠAAACGTA	GTAACAGAČG CATTGTCTGC	TGGATTTOTG
30351			CAGGTGTGAC GTCCACACTG		
30401			TTGGGTTTTG AACCCAAAAC		CAATATGCAA GTTATACGTT
30451			AAGGATTGAT TTCCTAACTA		
30501	ACTTGATGTT TGAACTACAA		TTGATGCTCA AACTACGAGT		
30551			ATAAACTCAG TATTTGAGTC		
30601			GTTTACAGCT CÀAATGTCGA		
30651			CCAAGGGGTT GGTTCCCCAA		
30701			GCGCTTGAAT CCCGAACTTA		
30751			AAAAATTGGC TTTTTAACCG		
30801			AACTAGGAAC TTGATCCTTG		
30851			AACAAAAATA TTGTTTTTAT		
30901			TAACTGTAGA ATTGACATCT		
30951			CAAAATGTGG GTTTTACACC		
31001	TTTCAGTTTT AAAGTCAAAA	GGCTGTTAAA CCGACAATTT	GGCAGTTTGG CCGTCAAACC	CTCCAATATC GAGGTTATAG	TGGAACAGTT ACCTTGTCAA
31051	CAAAGTGCTC GTTTCACGAG	ATCTTATTAT TAGAATAATA	AAGATTTGAC TTCTAAACTG	GAAAATGGAG CTTTTACCTC	TGCTACTAAA ACGATGATTT
31101	CAATTCCTTC GTTAAGGAAG	CTGGACCCAG GACCTGGGTC	AATATTGGAA TTATAACCTT	CTTTAGAAAT GAAATCTTTA	CCTCTAGAAT
31151	CTGAAGGCAC GACTTCCGTG	AGCCTATACA TCGGATATGT	AACGCTGTTG TTGCGACAAC	GATTTATGCC CTAAATACGG	TAACCTATCA ATTGGATAGT
31201	GCTTATCCAA CGAATAGGTT		TAAAACTGCC ATTTTGACGG		

Figure 27 AB

31251	AGTTTACTTA	AMEGGAGACA	AAACTAAACC	TGTAACACTA	ACCATTAGAC
	TCAAATGAAT	TTGCCTCTGT	TTTGATTTGG	ACATTGTGAT	TGGTAATGTG
31301	TAAACGGTAC ATTTGCCATG		GGAGACACAA CCTCTGTGTT		
31351	TCATTTTCAT AGTAAAAGTA		TGGCCACAAC ACCGGTGTTG		
31401	CACATCCTCT	TACACTTTTT	CATACATTGC	CCAAGAATAA	AGAATCGTTT
	GTGTAGGAGA	ATGTGAAAAA	GTATGTAACG	GGTTCTTATT	TCTTAGCAAA
31451	GTGTTATGTT CACAATACAA		TATTTTTCAA ATAAAAAGTT		
31501	TTTTTCATTC AAAAAGTAAG	ACTACTATAC TCATCATATC	CCCCACCACC	ACATAGCTTA TGTATCGAAT	TACAGATCAC ATGTCTAGTG
31551			AGAACCCTAG TCTTGGGATC		
31601	TCCCAACACA	CAGAGTACAC	AGTCCTTTCT	CCCCGGCTGG	CCTTAAAAAG
	AGGGTTGTGT	GTCTCATGTG	TCAGGAAAGA	GGGGCCGACC	GGAATTTTTC
31-651	CATCATATCA	TGGGTAACAG	ACATATTCTT	AGGTGTTATA	TTCCACACGG
	GTAGTATAGT	ACCCATTGTC	TGTATAAGAA	TCCACAATAT	AAGGTGTGCC
31701	TTTCCTGTCG	AGCCAAACGC	TCATCAGTGA	TATTAATAA	CTCCCCGGGC
	AAAGGACAGC	TCGGTTTGCG	AGTAGTCACT	TTTATTA	GAGGGGCCCG
31751	AGCTCACTTA	AGTTCATGTC	GCTGTCCAGC	TGCTGAGCCA	CAGGCTGCTG
	TCGAGTGAAT	TCAAGTACAG	CGACAGGTCG	ACGACTCGGT	GTCCGACGAC
31801	TCCAACTTGC AGGTTGAACG	GGTTGCTTAA CCAACGAATT	CGGGCGGCGA	AGGAGAAGTC TCCTCTTCAG	CACGCCTACA GTGCGGATGT
31851	TGGGGGTAGA	GTCATAATCG	TGCATCAGGA	TAGGGCGGTG	GTGCTGCAGC
	ACCCCCATCT	CAGTATTAGC	ACGTAGTCCT	ATCCCGCCAC	CACGACGTCG
31901	ACCGCGCGAA	TAAACTGCTG	GGGGGGGGGG	TCCGTCCTGC	AGGAATACAA
	TCGCGCGCTT	ATTTGACGAC	GGGGGGGGGGG	AGGCAGGACG	TCCTTATGTT
31951	CATGGCAGTG GTACCGTCAC	GTCTCCTCAG CAGAGGAGTC	CGATGATTCG GCTACTAAGC	CACCGCCCGC	AGCATAAGGC TCGTATTCCG
32001	GCCTTGTCCT	CCGGGCACAG	CAGCGCACCC	TGATCTCACT	TAAATCAGCA
	CGGAACAGGA	GGCCCGTGTC	GTCGCGTGGG	ACTAGAGTGA	ATTTAGTCGT
32051	CAGTAACTGC	AGCACAGCAC	CACAATATTG	TTCAAAATCC	CACAGTGCAA
	GTCATTGACG	TCGTGTCGTG	GTGTTATAAC	AAGTTTTAGG	GTGTCACGTT
32101	GGCGCTGTAT	CCAAAGCTCA	TGGCGGGGAC	CACAGAACCC	ACGTGGCCAT
	CCGCGACATA	GGTTTCGAGT	ACCGCCCCTG	GTGTCTTGGG	TGCACCGGTA
32151	CATACCACAA GTATGGTGTT	GCGCAGGTAG	ATTAAGTGGC TAATTCACCG	GACCCCTCAT CTGGGGAGTA	AAACACGCTG TTTGTGCGAC

Figure 27 AH

32201	GACATAAACA CTGTATTTGT	AATGGAGAAA	TGGCATGTTG ACCGTACAAC	TAATTCACCA ATTAAGTGGT	CCTCCC A GGAGGGCCAT
32251					ATCCTAAACC TAGGATTTGG
32301	AGCTGGCCAA TCGACCGGTT				ACCGGGACTG TGGCCCTGAC
32351					TCATCATGCT AGTAGTACGA
32401		TCAATGTTGG AGTTACAACC			
32451		AAGCTCCTCC TTCGAGGAGG			
32501		TCAGCGTAAA AGTCGCATTT			CTCGCACGTA GAGCGTGCAT
32551					AGCGGATGAT TCGCCTACTA
32601		GGTAGCGCGG CCATCGCGCC			
32651					GTCGTAGTGT CAGCATCACA
32701		GGAACGCCGG CCTTGCGGCC			
32751		GACAAACAGA CTGITTGTCT			
32801		TAGTTGTAGT ATCAACATCA			
32851		GGGTTCTATG CCCAAGATAC			
32901		CCGCAGAATA GGCGTCTTAT			
32951	CTGCGAGTCA GACGCTCAGT	CACACGGGAG GTGTGCCCTC	GAGCGGGAAG CTCGCCCTTC	AGCTGGAAGA TCGACCTTCT	ACCATGTTTT TGGTACAAAA
	TTTTTTTTTT AAAAAAAA				
33051	GTGAACGCGC CACTTGCGCG	TCCCCTCCGG AGGGGAGGCC	TGGCGTGGTC ACCGCACCAG	AAACTCTACA TTTGAGATGT	GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG				AAGGCAAACG TTCCGTTTGC

Figure 27 AI

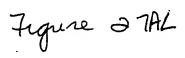
33151	GCCCTCACGT ( CGGGAGTGCA	GE CACCTG	GTAAAGGCTA CATTTCCGAT	AACCCTTCAG TTGGGAAGTC	egtgaat 70 CCACIII, ag
33201	CTCTATAAAC GAGATATTTG	ATTCCAGCAC TAAGGTCGTG			
33251	GCCACCTTCT CGGTGGAAGA	CAATATATCT GTTATATAGA			
33301	ATTGTAAAAA TAACATTTTT	TCTGCTCCAG AGACGAGGTC			
33351	AATCATGATT TTAGTACTAA	GCAAAAATTC CGTTTTTAAG			
33401		TTAACAAAAA AATTGTTTTT			
33451		ATAATCGTGC TATTAGCACG			
33501		CCATGACAAA GGTACTGTTT			
33551	CGGAGCTATG GCCTCGATAC	CTAACCAGCG GATTGGTCGC	TAGCCCCGAT ATCGGGGCTA	GTAAGCTTGT CATTCGAACA	TGCATGGGCG ACGTACCCGC
33601	GCGATATAAA CGCTATATTT	ATGCAAGGTG TACGTTCCAC	CTGCTCAAAA GACGAGTTTT	AATCAGGCAA TTAGTCCGTT	AGCCTCGCGC TCGGAGCGCG
33651	AAAAAAGAAA TTTTTTTCTTT	GCACATCGTA CGTGTAGCAT			
33701					AACATGTCTG TTGTACAGAC
33751					ATTTAAACAT TAAATTTGTA
33801	TAGAAGCCTG ATCTTCGGAC	TCTTACAACA AGAATGTTGT	GGAAAAACAA CCTTTTTGTT	CCCTTATAAG GGGAATATTC	CATAAGACGG GTATTCTGCC
33851	ACTACGGCCA TGATGCCGGT	TGCCGGCGTG ACGGCCGCAC	ACCGTAAAAA TGGCATTTTT	AACTGGTCAC TTGACCAGTG	CGTGATTAAA GCACTAATTT
33901	AAGCACCACC TTCGTGGTGG	GACAGCTCCT CTGTCGAGGA	CGGTCATGTC GCCAGTACAG	CGGAGTCATA GCCTCAGTAT	ATGTAAGACT TACATTCTGA
33951	CGGTAAACAC GCCATTTGTG	ATCAGGTTGA TAGTCCAACT	TTCACATCGG AAGTGTAGCC	TCAGTGCTAA AGTCACGATT	AAAGCGACCG TTTCGCTGGC
34001	AAATAGCCCG TTTATCGGGC	GGGGAATACA CCCCTTATGT	TACCCGCAGG	CGTAGAGACA GCATCTCTGT	ACATTACAGC TGTAATGTCG
34051	CCCCATAGGA GGGGTATCCT	GGTATAACAA CCATATTGTT	AATTAATAGG TTAATTATCC	AGAGAAAAAC TCTCTTTTTG	ACATAAACAC TGTATTTGTG

Figure 27AJ

34101	•	G. BACGGAT	CCGTTTTATC	GTGGGAGGC	GAGGTC
34151	ACATACAGCG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG	GGCAGCCATA CCGTCGGTAT	ACAGTCAGCC TGTCAGTCGG	TTACCAGTAA AATGGTCATT
	AAAAGAAAAC TTTTCTTTTG	GATAATTTTT	TTGTGGTGAG	CTGTGCCGTG	GTCGAGTTAG
		ATTTTTTCCC	GGTTCACGTC	TCGCTCATAT	ATATCCTGAT
34301	AAAAATGACG TTTTTTACTGC			AAACACCCAG TTTGTGGGTC	
34351				AAACCCACAA TTTGGGTGTT	
34401				CTTCCCATTT GAAGGGTAAA	
34451	ACAATTCCCA TGTTAAGGGT	ACACATACAA TGTGTATGTT	GTTACTCCGC CAATGAGGCG	CCTAAAACCT GGATTTTGGA	ACGTCACCCG TGCAGTGGGC
34501	CCCCGTTCCC GGGGCAAGGG	ACGCCCCGCG TGCGGGGGCGC	CCACGTCACA GGTGCAGTGT	AACTCCACCC TTGAGGTGGG	CCTCATTATC GGAGTAATAG
					PacI
34551	ATATTGGCTT TATAACCGAA	CAATCCAAAA GTTAGGTTTT	TAAGGTATAT ATTCCATATA	TATTGATGAT ATAACTACTA	GTTAATTAAG CAATTAATTC
34551 34601	TATAACCGAA	GTTAGGTTTT TGCGACGCGA	ATTCCATATA GGCTGGATGG	TATTGATGAT ATAACTACTA CCTTCCCCAT GGAAGGGGTA	CAATTAATTC
	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG	ATAACTACTA CCTTCCCCAT	CAATTAATTC  TATGATTCTT ATACTAAGAA  TGCTGTCCAG
34601	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA . TTGCAGGCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA
34601 34651	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  GGAACCGTAA CCTTGGCATT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  GGAACCGTAA CCTTGGCATT  CCTGACGAGGC	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751 34801	TATAACCGAA  AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CGTACCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTAG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT TAAAGATACC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGCTGCGAGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG
34601 34651 34701 34751 34801 34851	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CGTACGAGC CCTGACGAGC GGACTGCTCG GACAGGACTA CTTGCCTGT CTGTCCTGAT CTGTCCTGT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCTAGCC GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG CCGCTTACCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC ACGGAGCCACG
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC  GCAGGTAGAT CGTCCATCTA  CGTCCATCTA  CCTGACGAGC GGACTGCTCG  GACAGGACTA  CTGTCCTGT  CTGTCCTGAT  CCTCCTGAT  CTGTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGT  CGAGAGGACAA  CCTTCCGGGAA	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGGAC GCGTGGCGCT	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG CCGCTTACCG GGCGAATGGC TTCTCATAGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC CGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG CGCGAAACCC CCGCTTTGGG TCCCTCGTGC ACGGACCACG CGCCTTTCTC CGCGAAACAG

Figure 27 AK

35051	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	TGAGTCCĂAC
			AATAGGCCAT		
35101	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA CGGTGACCGT	GCAGCCACTG CGTCGGTGAC	GTAACAGGAT CATTGTCCTA
35151	TAGCAGAGCG	AGGTATGTAG TCCATACATC	GCGGTGCTAC CGCCACGATG	TCTCAAGAAC	TTCACCACCG
35201	<b>C</b> である C でる C C C	<b>C</b> であいる	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG
33201	GATTGATGCC	GATGTGATCT	TCCTGTCATA	AACCATAGAC	GCGAGACGAC
35251	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA
0020-	TTCGGTCAAT	GGAAGCCTTT	TTCTCAACCA	TCGAGAACTA	GGCCGTTTGT
35301	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC
	TTGGTGGCGA	CCATCGCCAC	CAAAAAAAACA	AACGTTCGTC	GTCTAATGCG
35351	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT
	CGTCTTTTTT	TCCTAGAGTT	CTTCTAGGAA	ACTAGAAAAG	ATGCCCCAGA
35401	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT
	CTGCGAGTCA	CCTTGCTTTT	GAGTGCAATT	CCCTAAAACC	AGTACTCTAA
35451	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATCAATCT	AAAGTATATA
	TAGTTTTTCC	TAGAAGTGGA	TCTAGGAAAA	TTTAGTTAGA	TTTCATATAT
35501	TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	GAGGCACCTA
	ACTCATTTGA	ACCAGACTGT	CAATGGTTAC	GAATTAGTCA	CTCCGTGGAT
35551	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC
	AGAGTCGCTA	GACAGATAAA	GCAAGTAGGT	ATCAACGGAC	TGAGGGGCAG
35601	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC
				•	GGTCACGACG
35651	AATGATACCG	CGAGACCCAC	GCTCACCGGC	TCCAGATTTA	TCAGCAATAA
	TTACTATGGC	GCTCTGGGTG	CGAGTGGCCG	AGGTCTAAAT	AGTCGTTATT
35701	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCCTGC	AACTTTATCC
				_	TTGAAATAGG
35751	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	GAAGCTAGAG	TAAGTAGTTC
	CGGAGGTAGG	TCAGATAATT	, YYCYYCGCC	CTTCGATCTC	ATTCATCAAG
35801	GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG
					CCGTAGCACC
35851	TGTCACGCTC	GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA
				•	AAGGGTTGCT
35901	TCAAGGCGAG	TTACATGATO	CCCCATGTTC	TGCAAAAAA	CGGTTAGCTC
	AGTTCCGCTC	AATGTACTAG	GGGGTACAAC	: ACGTTTTTT(	CCCAATCGAG
35951	CTTCGGTCCT	CCGATCGTTC	TCAGAAGTA	GTTGGCCGC	A GTGTTATCAC
	GAAGCCAGGA	GGCTAGCAAC	AGTCTTCAT	CAACCGGCG'	r cacaatagig



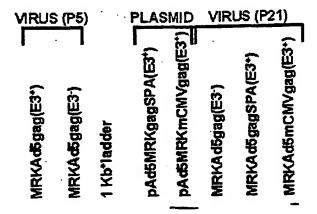
WO 02/22080 PCT/US01/28861 36001 TCATGGTTAT SAAGCACTG CATAATTCTC TTACTGTCAT GCCATCATA AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT 36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA CGGGATAATA CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT 36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA 36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA 36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT 36301 - GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA CGCAAAGACC CACTCGTTTT TGTCCTTCCG TTTTACGGCG TTTTTTCCCT 36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA TATTCCCGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT 36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC 36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT 36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT 36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

## PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34) TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM



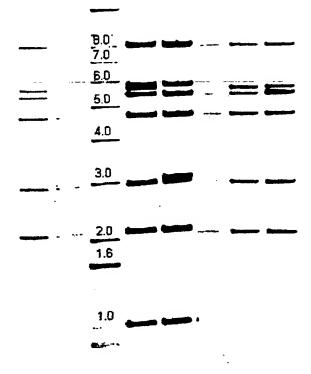


FIGURE 28

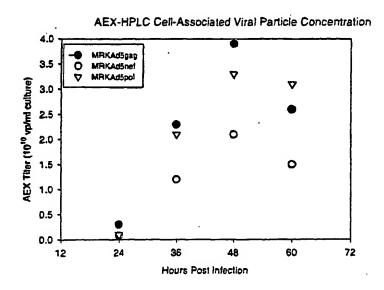


FIGURE 29A

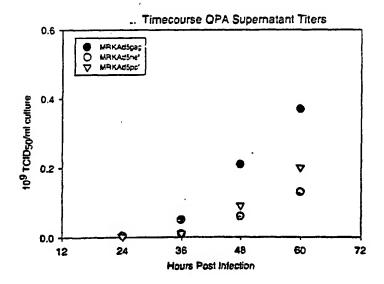


FIGURE 29B

PCT/US01/28861 WO 02/22080

atg Met 1	gat Asp	gca Ala	atg Met	aag Lys 5	aga Arg	ggg ggg	ctc Leu	tgc Cys	tgt Cys 10	gtg Val	ctg Leu	ctg Leu	ctg Leu	tgt Cys 15	gga Gly	<b>48</b>
gca Ala	gtc Val	ttc Phe	gtt Val 20	tcg Ser	ccc Pro	agc Ser	gag Glu	atc Ile 25	tcc Ser	att Ile	gtg Val	tgg Trp	gcc Ala 30	tcc Ser	agg Arg	96
gag Glu	ctg Leu	gag Glu 35	agg Arg	ttt Phe	gct Ala	gtg Val	aac Asn 40	cct Pro	ggc Gly	ctg Leu	ctg Leu	gag Glu 45	acc Thr	tct Ser	gag Glu	144
GJA aaa	tgc Cys 50	agg Arg	cag Gln	atc Ile	ctg Leu	ggc Gly 55	cag Gln	ctc Leu	cag Gln	ccc Pro	tcc Ser 60	ctg Leu	caa Gln	aca Thr	ggc	192
tct Ser 65	gag Glu	gag Glu	ctg Leu	agg Arg	tcc Ser 70	ctg Leu	tac Tyr	aac Asn	aca Thr	gtg Val 75	gct Ala	acc Thr	ctg Leu	tac Tyr	tgt Cys 80	240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac Asp	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
att Ile	gag Glu	gag Glu	gag Glu 100	cag Gln	aac Asn	aag Lys	tcc Ser	aag Lys 105	aag Lys	aag Lys	gcc Ala	cag Gln	cag Gln 110	gct Ala	gct Ala	336
gct Ala	ggc Gly	aca Thr 115	ggc Gly	aac Asn	tcc Ser	agc Ser	cag Gln 120	gtg Val	tcc Ser	cag Gln	aac Asn	tac Tyr 125	ccc Pro	att Ile	gtg Val	384
cag Gln	aac Asn 130	ctc Leu	cag Gln	Gly	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	ccc Pro	cgg Arg	acc Thr	432
ctg Leu 145	aat Asn	gcc Ala	tgg Trp	gtg Val	aag Lys 150	gtg Val	gtg Val	gag Glu	gag Glu	aag Lys 155	gcc Ala	ttc Phe	tcc Ser	cct Pro	gag Glu 160	480
gtg Val	atc Ile	CCC	atg Met	ttc Phe 165	tct Ser	gcc Ala	ctg Leu	tct Ser	gag Glu 170	ggt Gly	gcc Ala	acc Thr	ccc Pro	cag Gln 175	gac Asp	528
ctg Leu	aac Asn	acc Thr	atg Met 180	Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	GIA	cat His	cag Gln	gct Ala	gcc Ala 190	atg Met	cag Gln	576
atg Met	ctg	aag Lys 195	Glu	acc Thr	atc	aat Asn	gag Glu 200	GIU	gct	gct Ala	gag Glu	tgg Trp 205	ASP	agg Arg	ctg Leu	624
cat	cct Pro 210	Val	cac	gct Ala	ggc	Pro 215	ITE	gcc	Pro	ggc	Gln 220	Met	agg Arg	gag Glu	Pro	672
agg Arg 225	Gly	tct Ser	gac Asp	att Ile	gct Ala 230	Gly	acc Thr	acc Thr	tcc Ser	Thr 235	Den	cag Gln	gag Glu	cag Gln	att Ile 240	720
ggc Gly	tgg Trp	atg Met	acc Thr	Asr 245	Asn	Pro	ccc Pro	ato Ile	e cct Pro 250	) Agr	Gly ggg	g gaa g Glu	ato Ile	tac Tyr 255	aag Lys	768

Figure 30 A^{*}

agg tgg a Arg Trp I	atc atc ct lle lle Le 260	g ggc ctg eu Gly Leu	aac aag Asn Lys 265	att gtg Ile Val	agg atg Arg Met	tac tcc Tyr Ser 270	Pro	816
Thr Ser I	atc ctg ga [le Leu As 275	c atc agg p Ile Arg	cag ggc Gln Gly 280	ccc aag Pro Lys	gag ccc Glu Pro 285	ttc agg Phe Arg	gac Asp	864
tat gtg g Tyr Val A 290	pac agg tt Asp Arg Ph	c tac aag e Tyr Lys 295	acc ctg Thr Leu	agg gct Arg Ala	gag cag Glu Gln 300	gcc tcc Ala Ser	cag Gln	912
gag gtg a Glu Val L 305	ag aac to ys Asn Ti	g atg aca p Met Thr 310	gag acc Glu Thr	ctg ctg Leu Leu 315	gtg cag Val Gln	aat gcc Asn Ala	aac Asn 320	960
cct gac t Pro Asp C	gc aag ac lys Lys Th 32	c atc ctg r Ile Leu 5	aag gcc Lys Ala	ctg ggc Leu Gly 330	cct gct Pro Ala	gcc acc Ala Thr 335	Leu	1008
gag gag a Glu Glu M	atg atg ac Met Th 340	a gcc tgc ir Ala Cys	cag ggg Gln Gly 345	gtg ggg Val Gly	ggc cct Gly Pro	ggt cac Gly His 350	aag Lys	1056
Ala Arg V	stg ctg go Val Leu Al 855	t gag gcc a Glu Ala	atg tcc Met Ser 360	cag gtg Gln Val	acc aac Thr Asn 365	tcc gcc Ser Ala	acc Thr	1104
atc atg a Ile Met M 370	itg cag ag Met Gln Ar	g ggc aac g Gly Asn 375	ttc agg Phe Arg	aac cag Asn Gln	agg aag Arg Lys 380	aca gtg Thr Val	aag Lys	1152
tgc ttc a Cys Phe A 385	ac tgt gg Asn Cys Gl	y Lys Val 390	ggc cac Gly His	att gcc Ile Ala 395	aag aac Lys Asn	tgt agg Cys Arg	gcc Ala 400	1200
ccc agg a Pro Arg L	ys Lys Gl	c tgc tgg y Cys Trp 5	aag tgt Lys Cys	ggc aag Gly Lys 410	gag ggc Glu Gly	cac cag His Gln 415	atg Met	1248
aag gac t Lys Asp C	gc aat ga lys Asn Gl 420	g agg cag u Arg Gln	gcc aac Ala Asn 425	ttc ctg Phe Leu	ggc aaa Gly Lys	atc tgg Ile Trp 430	CCC Pro	1296
Ser His L	ag ggc ag ys Gly Ar 35	g cct ggc g Pro Gly	aac ttc Asn Phe 440	ctc cag Leu Gln	tcc agg Ser Arg 445	cct gag Pro Glu	ccc Pro	1344
aca gcc c Thr Ala P 450	ct ccc ga Pro Pro Gl	g gag tcc u Glu Ser 455	ttc agg Phe Arg	Phe Gly	Glu Glu	aag acc Lys Thr	acc Thr	1392
ccc agc c Pro Ser G 465	ag aag ca Sln Lys Gl	g gag ccc n Glu Pro 470	att gac Ile Asp	aag gag Lys Glu 475	ctg tac Leu Tyr	ccc ctg Pro Leu	gcc Ala 480	1440
tcc ctg a Ser Leu A	gg tcc ct rg Ser Le 48	g ttt ggc n Phe Gly	aac gac Asn Asp	ccc tcc Pro Ser 490	tcc cag Ser Gln	taa (SI * (SI	D NO:36) D NO:37)	1482

Figure 30 B

Figure 31

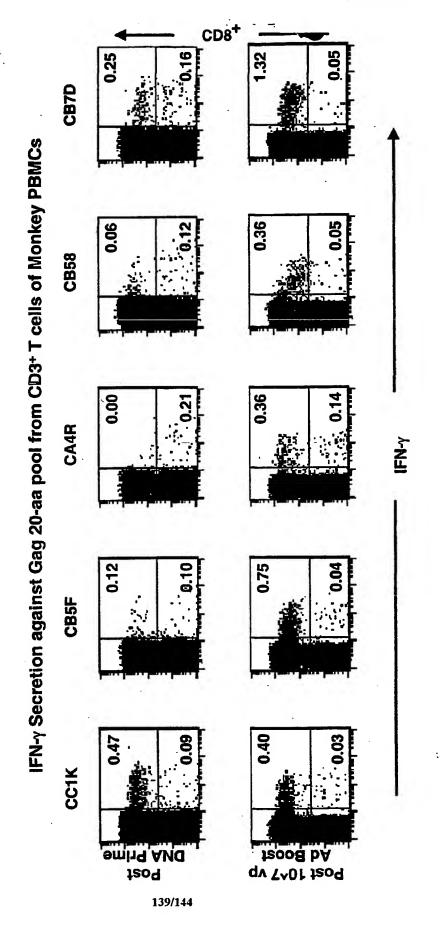
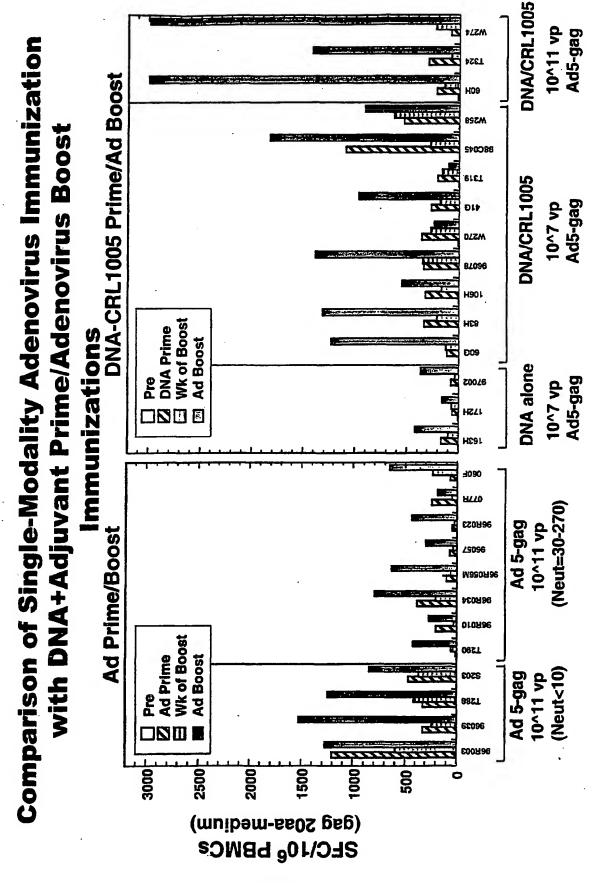


FIGURE 32



# FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
	ACATCAGGCA				
	TGAGGGCTGA				
	AGAATGCCAA				
	AGGAGATGAT				
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
	ACTGTAGGGC				
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
	GGCCTGGCAA				
	GGTTTGGGGA				
	ACCCCCTGGC				
	TCTCCCCCAT				
	AGCAGTGGCC				
	AGAAGGAGGG				
					GGACTTCAGG
					CCACCCCGCT
					CTTCTCTGTG
					CAACAATGAG
					CTCCCTGCC
					CCCTGACATT
					TGGGCAGCAC
					CACCCTGAC
					CCCCGACAAG
					TGACATCCAG
					GGTGAGGCAG
					GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

## FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCITCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACTT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA SEO ID NO: 38

## FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Glu Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

## FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

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